U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-252 007

EFFECTS OF MIREX, METHOXYCHLOR, AND MALATHION ON DEVELOPMENT OF CRABS

DUKE UNIVERSITY

PREPARED FOR
ENVIRONMENTAL PROTECTION AGENCY

MARCH 1976

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-76-007
2.
3. RECIPIENT'S ACCESSION NO. PB-252 007
4. TITLE AND SUBTITLE EFFECTS OF MIREX, METHOXYCHLOR, AND MALATHION ON DEVELOPMENT OF CRABS
5. REPORT DATE March 1976 (Issuing Date)
6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Cazlyn G. Bookhout and John D. Costlow, Jr.
8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS

Duke University Marine Laboratory
Beaufort, North Carolina 28516

10. PROGRAM ELEMENT NO. 1EA077;ROAP 10AKC;Task 37
11. CONTRACT/GRANT NO. R-801128-02-2
12. SPONSORING AGENCY NAME AND ADDRESS

U.S. Environmental Protection Agency
Office of Research and Development
Environmental Research Laboratory
Gulf Breeze, Florida 32561

13. TYPE OF REPORT AND PERIOD COVERED 10/72 to 4/75
14. SPONSORING AGENCY CODE EPA-ORD
15. SUPPLEMENTARY NOTES
16. ABSTRACT

Laboratory experiments were conducted to determine the effects of mirex, methoxychlor and malathion on the larval development of *Callinectes sapidus* from the time of hatching until the first crab stage is reached. For comparison, similar investigations were made to ascertain the effects of methoxychlor and malathion on larval development of *Rhithropanopeus harrisii*.

The effect of a range of concentrations of each insecticide on survival of larvae of *C. sapidus* and *R. harrisii* was determined, as well as concentrations which were sublethal and lethal. Zoeal and total development to the first crab stage of *R. harrisii* and *C. sapidus* was prolonged in relation to increased concentrations of methoxychlor and malathion. Other sublethal effects of methoxychlor and malathion included abnormal development of the pleopods of male *R. harrisii* and male *C. sapidus* early crab stages, and autotomy of the legs of *R. harrisii* megalopa and early crab stages. The developmental stages in which larvae are particularly sensitive vary in the two species and with the three insecticides. Mirex residues of *C. sapidus* larvae reared in different concentrations of mirex, and methoxychlor residues of *R. harrisii* and *C. sapidus* larvae reared in concentrations of methoxychlor were determined.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pesticides Bioassay Crustacea Crabs Malathion	Insecticide toxicity Mirex Methoxychlor Blue crabs Mud crabs Larval development	6F

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 96
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE 5.00

EPA Form 2220-1 (9-73)

☆ U. S. GOVERNMENT PRINTING OFFICE: 1976-657-695/5389 Region No. 5-11

124147

EPA-600/3-76-007
March 1976

PB 252 007

Ecological Research Series

EFFECTS OF MIREX, METHOXYCHLOR, AND MALATHION ON DEVELOPMENT OF CRABS

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-76-007
March 1976

EFFECTS OF MIREX, METHOXYCHLOR, AND MALATHION ON DEVELOPMENT OF CRABS

by

Cazlyn G. Bookhout and
John D. Costlow, Jr.
Duke University
Beaufort, North Carolina 28516

Grant No. R-801128-02-2

Project Officer

Jack Lowe
Gulf Breeze Environmental Research Laboratory
Gulf Breeze, Florida 32561

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
GULF BREEZE ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561

ia

DISCLAIMER

This report has been reviewed by the Gulf Breeze Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise, and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The Environmental Research Laboratory, Gulf Breeze contributes to this multidisciplinary focus through a research program which emphasizes the ecological effects on the marine environment of pesticides and other organic and inorganic pollutants. Specific research outputs include

- information essential for the EPA pesticide registration and control program
- information essential for the development of the EPA Water Quality Criteria
- toxic effects of pollutants, singly and in combination, on marine and estuarine organisms
- effects of pollutants, singly and in combination, on the physiology and development of marine and estuarine organisms
- assessment of the hazards of human exposure to pollutants which reach man through bioconcentration in marine and estuarine food chains
- impact on marine and estuarine ecosystems of offshore dumping of petrochemicals

This report describes the effects of three insecticides on the development of crabs. The data will be useful in establishing estuarine water quality criteria for mirex, methoxychlor, and malathion.

Thomas W. Duke
Director
Environmental Research
 Laboratory, Gulf Breeze

ABSTRACT

Laboratory experiments were conducted to determine the effects of mirex, methoxychlor and malathion on the larval development of Callinectes sapidus from the time of hatching until the first crab stage is reached. For comparison, similar investigations were made to ascertain the effects of methoxychlor and malathion on larval development of Rhithropanopeus harrisii.

The effect of a range of concentrations of each insecticide on survival of larvae of C. sapidus and R. harrisii was determined, as well as concentrations which were sublethal and lethal. Zoeal and total development to the first crab stage of R. harrisii and C. sapidus was prolonged in relation to increased concentrations of methoxychlor and malathion. Other sublethal effects of methoxychlor and malathion included abnormal development of the pleopods of male R. harrisii and male C. sapidus early crab stages, and autotomy of the legs of R. harrisii megalopa and early crab stages. The developmental stages in which larvae are particularly sensitive vary in the two species and with the three insecticides. Mirex residues of C. sapidus larvae reared in different concentrations of mirex, and methoxychlor residues of R. harrisii and C. sapidus larvae reared in concentrations of methoxychlor were determined.

This report was submitted in fulfillment of Grant No. R-801-128-02-2 by Duke University under the sponsorship of the Environmental Protection Agency. Work was completed as of October 15, 1975.

CONTENTS

Section		Page
I	General Introduction	1
II	Conclusions	4
III	Recommendations	7
IV	General Materials and Methods	9
V	Mirex	16
	Introduction	16
	Results	18
	Discussion	24
VI	Methoxychlor	30
	Introduction	30
	Results	32
	Discussion	48
VII	Malathion	53
	Introduction	53
	Results	55
	Discussion	69
VIII	General Discussion	73
IX	References	78
X	Glossary	84

FIGURES

Page

No.

1. Average percent survival of two replicate series of Callinectes sapidus larvae, Cs II and Cs VI, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of mirex. 20

2. Average percent survival of four replicate series of Rhithropanopeus harrisii larvae, Rh I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of methoxychlor. 35

3. Average percent survival of four replicate series of Callinectes sapidus larvae, Cs I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of methoxychlor. 42

4. Average percent survival of four replicate series of Rhithropanopeus harrisii, Rh I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of malathion. 57

5. Average percent survival of four replicate series of Callinectes sapidus, Cs I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of malathion. 64

LIST OF TABLES

Table	Title	Page
1.	EFFECT OF MIREX ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Callinectes sapidus* II AND VI	19
2.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MIREX ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* II AND VI	21
3.	MEAN PERCENT SURVIVAL TO MEGALOPA AND TO FIRST CRAB IN *C. sapidus* II AND VI	22
4.	PERCENT MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* II AND VI	23
5.	RESIDUES OF DDE, DDD AND DDT IN FIVE MOTHER CRABS	24
6.	MIREX RESIDUES IN BLUE CRABS AND THEIR LARVAE	25
7.	EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Rhithropanopeus harrisii* I-IV	34
8.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV	36
9.	AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV	36
10.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV	37
11.	PERCENT MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV	38

LIST OF TABLES (continued)

Table	Title	Page
12.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV	39
13.	METHOXYCHLOR RESIDUES IN *R. harrisii* LARVAE	40
14.	EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Callinectes sapidus* I-IV	41
15.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV	43
16.	AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMEPNT OF *C. sapidus* I-IV	44
17.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON DURATION OF ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV	44
18.	PERCENT MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV	46
19.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV	47
20.	METHOXYCHLOR RESIDUES IN *C. sapidus* LARVAE	48
21.	EFFECT OF MALATHION ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Rhithropanopeus harrisii* I-IV	56
22.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV	58
23.	AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV	58

LIST OF TABLES (continued)

Table	Title	Page
24.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV	59
25.	PERCENT MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV	60
26.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV	61
27.	RESIDUES IN FOUR MOTHER CRABS, Rh I-IV	62
28.	EFFECT OF MALATHION ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Callinectes sapidus* I-IV	63
29.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV	65
30.	AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV	66
31.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV	66
32.	PERCENT MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV	67
33.	TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV	68

ACKNOWLEDGMENTS

The help of the following persons has been invaluable and is gratefully acknowledged. Dr. Marit Christiansen, University of Oslo, Oslo, Norway, assisted in technical aspects of the research and in discussing topics relevant to the project. Dr. Genevieve Payen, University of Paris, Paris, France, did the research on the effect of methoxychlor and malathion on the external and internal sexual characters of *Rhithropanopeus harrisii* and *Callinectes sapidus*. Dr. Robert Monroe, Department of Experimental Statistics, North Carolina State University, Raleigh, North Carolina, served as statistical consultant.

The technical assistance of Mrs. Charles Johnson throughout the project and the untiring efforts of Ms. Mamre Wilson in typing the manuscript are greatly appreciated.

SECTION I

GENERAL INTRODUCTION

Crabs are phylogenetically related to insects, and juvenile crabs show extreme sensitivity to certain insecticides. Crabs in larval stages might be expected to be more sensitive than juveniles, and, therefore, more valuable in establishing safe levels of insecticides in estuaries. There are relatively few papers in the literature, however, on the effects of different concentrations of pesticides on the larval development of crabs.

Buchanan *et al*. (1970) studied the effect of Sevin, a carbamate, on the survival and development of larvae in the first zoeal stage of the dungeness crab, *Cancer magister*. They found that the change from sublethal to acute toxicity occurred between 3.2 mg/l (milligrams per liter - parts per million) and 10.0 mg/l, and that duration of the first larval stage increased with concentration. Larvae in first zoeal stages were more sensitive to Sevin than juveniles, and juveniles more sensitive than adults.

Epifanio (1971) made a thorough study of the effects of various concentrations of dieldrin on the larval development of two species of xanthid crabs, *Leptodius floridanus* and *Panopeus herbstii*. He reported that concentrations of dieldrin in seawater of 1.0 ppb (parts per billion = µg/l micrograms per liter) or higher are deleterious to xanthid crab larvae in the laboratory. Early stage larvae were more sensitive than larvae in later stages, and *L. floridanus* larvae were more sensitive to dieldrin than *P. herbstii* larvae. *Leptodius floridanus* larvae accumulated dieldrin 19.1 times as fast from 0.5 ppb in seawater as from 213.0 ppb in food. This, he believed, was because the pumping rate was much higher than the feeding rate (Epifanio, 1973).

Courtenay and Roberts (1973) conducted laboratory studies to determine lethal limits (96 hr - TL_{50}, tolerance limit) for toxaphene, temperature, salinity and their interaction effects on developmental stages of four species of decapods.

The bioassay organisms were the blue crab, Callinectes sapidus; the pink shrimp, Penaeus duorarum; the drift line crab, Sesarma cinereum; and the mud-crab, Rhithropanopeus harrisii. The decapod larvae became more tolerant to toxaphene in later developmental stages than earlier stages. Larvae of C. sapidus, S. cinereum and R. harrisii exposed to high concentrations of toxaphene showed contraction of the intestine and progressive vacuolation and necrosis of the hepatopancreas.

From the literature cited, it appears that larvae are more sensitive to insecticides than juveniles (Buchanan et al., 1971) and larvae in early stages are more sensitive than those in later stages (Epifanio, 1971; Courtenay and Roberts, 1973). There are so few publications on species in which the effects of insecticides on larvae and juveniles are known, however, that it is premature to make generalizations.

Most of the data on the effects of pesticides on aquatic organisms concern short-term effects with lethal concentrations. Long-term chronic exposure to low concentrations of an insecticide would be closer to what larvae or juveniles would receive in the field and the effects on organisms in the laboratory and field might be similar. When the larvae of the mud-crab, Rhithropanopeus harrisii, and the stone crab, Menippe mercenaria, were reared from the time of hatching to the first crab stage in a range of concentrations of mirex, each species showed different sublethal effects, but only slight differences in susceptibility to the same concentration of mirex (Bookhout et al., 1972). There is no information in the literature, however, on the effect of mirex on the development of the commercial blue crab, Callinectes sapidus, from the time of hatching to the first crab stage, nor is there information on the effect of methoxychlor or malathion on the complete larval development of any crab.

In the current investigation, the objective will be to determine the limits of concentrations of mirex, methoxychlor and malathion within which the blue crab, Callinectes sapidus can be reared from the time of hatching to the first crab stage. For comparison, similar chronic tests will be made to determine the concentrations of methoxychlor and malathion in which the mud-crab, Rhithropanopeus harrisii, will develop. From these studies, it should be possible to determine sublethal effects, the most sensitive larval stages and the sublethal and acutely toxic concentrations of the three common insecticides. From the data obtained in this investigation and from chronic tests on juvenile and adult crabs by other investigators, it should be possible to set more reliable standards of water quality for estuaries.

SECTION II

CONCLUSIONS

1. Concentrations of mirex from 0.01 ppb to 10.0 ppb have no effect on mortality of *Callinectes sapidus* larvae for five days after hatching. Thereafter, there is differential survival in relation to concentration. Concentrations of 0.01 ppb and 0.1 ppb mirex are sublethal and 1.0 ppb and 10.0 ppb acutely toxic to larvae.

2. There is no statistical difference in duration from hatching to megalopa, or hatching to 1st crab stage, in *C. sapidus* larvae reared in acetone control and in 0.01 ppb and 0.1 ppb mirex.

3. Significantly greater mortality of larvae occurs in zoeal stages III and VII and megalopa of *C. sapidus* in 0.1 ppb mirex, in zoeal stages II and III in 1.0 ppb mirex, and in zoeal stages I and II in 10.0 ppb mirex than in other stages.

4. *Callinectes sapidus* larvae reared in a range of concentrations of mirex show increased residues with concentration, but biological magnification of mirex is greatest in larvae reared in 0.01 ppb and decreases with concentrations ranging from 0.01 ppb to 10.0 ppb.

5. The range of concentrations of methoxychlor in which differential survival of *Rhithropanopeus harrisii* occurs from the time of hatching to the first crab stage is from 1.0 ppb to 7.0 ppb, whereas the range for similar development of *Callinectes sapidus* is from 0.7 ppb to 1.9 ppb. Concentrations of 1.0 ppb, 2.5 ppb, 4.0 ppb and 5.5 ppb are sublethal and 7.0 ppb methoxychlor is acutely toxic to *R. harrisii* larvae. Sublethal concentrations of methoxychlor for *C. sapidus* development are 0.7 ppb and 1.0 ppb, whereas 1.3 ppb, 1.6 ppb and 1.9 ppb are acutely toxic.

6. The total time from hatching to 1st crab stage in R. harrisii and C. sapidus is prolonged with each concentration primarily due to effects of methoxychlor on zoeal development. The reduction in molting rate is considered a sublethal effect of methoxychlor on both R. harrisii and C. sapidus zoeae. During sexual morphogenesis from megalopa to the 6th crab stage of R. harrisii and C. sapidus, methoxychlor has no apparent effect on the development of the gonads and their ducts, but has sublethal effects on the male pleopods.

7. In 1.0 ppb, 2.5 ppb and 4.0 ppb methoxychlor, stage I zoeae of R. harrisii are significantly sensitive as are zoeae of stages I and II to 5.5 ppb and 7.0 ppb. In the acutely toxic concentrations of 1.3 ppb, zoeae of stages II and III of C. sapidus are significantly sensitive, as are zoeae of stage II to 1.6 ppb and zoeae of stages I and II to 1.9 ppb.

8. Biological magnification of methoxychlor residues at 5 days and 10-15 days is much greater in C. sapidus than in R. harrisii. There is no evidence from the limited data that residues at any one concentration increases with age.

9. The range of concentrations in which differential survival of Rhithropanopeus harrisii occurs from the time of hatching to the 1st crab stage is 0.011 ppm to 0.02 ppm malathion. Two of the four concentrations are sublethal, 0.011 ppm and 0.014 ppm, and two are acutely toxic, 0.017 ppm and 0.02 ppm, to developmental stages of R. harrisii. Larvae reared in 0.05 ppm malathion do not survive beyond the 2nd zoeal stage. The range for similar development of Callinectes sapidus is from 0.02 ppm to 0.11 ppm malathion. Concentrations of 0.02 ppm and 0.05 ppm are sublethal and 0.08 ppm and 0.11 ppm are acutely toxic.

10. The average duration of zoeal development and total time from hatching to 1st crab stage is shortest in acetone control and is lengthened with each increase in concentration of malathion in R. harrisii from 0.011 ppm to 0.02 ppm and in C. sapidus from 0.02 ppm to 0.11 ppm. Other sublethal effects include abnormal development of the pleopods in the first three crab stages of both species and autotomy of legs in the megalopa and crab stages in R. harrisii.

11. In the development of R. harrisii from hatching to the 1st crab stage, zoeae in stage II and megalopa are significantly sensitive to 0.011 ppm malathion, as are zoeae in stage II to 0.014 ppm, 0.017 ppm and 0.02 ppm and zoeae in stage I to 0.05 ppm. In the larval development of C. sapidus, zoeae in stages II and III are significantly sensitive to 0.05 ppm malathion, as are zoeae in stages I, II and III to 0.07 ppm and zoeae in stage I to 0.11 ppm malathion.

SECTION III

RECOMMENDATIONS

1. To establish meaningful water quality standards for estuaries, the long-term effects of low concentrations of insecticides on the complete larval development of a number of marine organisms, including shrimp and crabs, should be considered, as well as chronic effects of insecticides on juveniles and adults of the same species.

2. Estuaries should be monitored for background levels of mirex and methoxychlor when the following conditions apply: when mirex is sprayed or applied directly to land areas to control fire ants and these areas are near estuaries, and when estuaries can receive drainage from areas such as fruit orchards, which are sprayed repeatedly with methoxychlor. Marsh areas, which are sprayed repeatedly to kill mosquito larvae, should be monitored for background levels of malathion after each spraying.

3. The current report outlined the effects of three insecticides on the development of crabs when the larvae were reared under optimum temperature and salinity conditions. Further investigation should be made to determine the effect of these insecticides on the complete larval development of crabs when the larvae are reared in a range of salinities and temperatures.

4. Methods should be developed to make accurate residue analyses of chlorinated hydrocarbon insecticides from samples of larvae weighing much less than 0.20 g wet weight. In the current project, it was a time consuming task to obtain 0.20 g of larvae in low concentrations of an insecticide and impossible to obtain late stages in high concentrations. Replicate analyses of larvae for residues of methoxychlor should be made as a supplement to those given in this report.

5. Micro methods for measurement of the enzyme acetylcholinesterase should be developed, so this test could be applied to crab larvae reared in different concentrations of malathion.

SECTION IV

GENERAL MATERIAL AND METHODS

Preliminary experiments were conducted to determine the range of concentrations of mirex, methoxychlor and malathion to use in the definitive chronic studies on the effect of mirex on the development of *Callinectes sapidus* and methoxychlor and malathion on the development of *Rhithropanopeus harrisii* and *Callinectes sapidus*.

Pesticide analytical grade acetone was used as a carrier for mirex, methoxychlor and malathion, because in preliminary studies it did not affect larval development of crabs. There was no significant difference in survival of larvae reared in seawater and 1.0 ppt (parts per thousand) insecticide grade acetone. Acetone control for experiments with each of the three pesticides was prepared by adding 1 ml of full strength acetone of insecticide grade to 999 ml of 20‰ filtered seawater for *R. harrisii* larvae or 30‰ filtered seawater for *C. sapidus* larvae to give a final concentration of 1.0 ppt.

The source and purity of the three insecticides used by Hazleton Laboratories America, Inc., to make stock solutions for this project is given below. Mirex (dodecachlorooctahydro-1,3,4-metheno-14-cyclobuta[cd]mentalene) was obtained from Allied Chemical Corporation and had a purity of 100%. Methoxychlor (1,1,1-trichlor-2,2-bis p-methoxyphenyl ethane) was secured from E. I. duPont de Nemours Company and had a purity of 99%. Malathion (0,0-dimethyl phosphoro-dithionate of diethyl mercapto-succinate) was obtained from American Cyanamid Company and had a purity of 96%.

All of the above were prepared in the following manner: known weight was dissolved in pesticide analytical grade acetone and various concentrations were made up from this stock solution.

One ml of each stock solution in concentrations of 0.01 ppm, 0.1 ppm, 1.0 ppm and 10.0 ppm mirex was added to 999 ml of

30‰ filtered seawater daily to give the final concentrations of 0.01 ppb to 10.0 ppb used to determine the effects of mirex on the development of Callinectes sapidus.

For experiments on the effect of methoxychlor on Rhithropanopeus harrisii development, one ml of stock solutions 1.0 ppm, 2.5 ppm, 4.0 ppm, 5.5 ppm and 7.0 ppm was added to 999 ml of 20‰ filtered seawater daily to give final concentrations of 1.0 ppb to 7.0 ppb methoxychlor.

For experiments on the effects of methoxychlor on C. sapidus, one ml of stock solutions of 0.7 ppm, 1.0 ppm, 1.3 ppm, 1.6 ppm and 1.9 ppm was added to 999 ml of 30‰ filtered seawater daily to give final concentrations of 0.7 ppb to 1.9 ppb.

For experiments on the effects of malathion on R. harrisii, one ml of stock solutions of 0.011 ppt, 0.014 ppt, 0.017 ppt, 0.02 ppt, and 0.05 ppt malathion was added to 999 ml of 20‰ filtered seawater daily to give final concentrations of 0.011 ppm to 0.05 ppm malathion.

For experiments on the effects of malathion on C. sapidus, one ml of stock solutions of 0.02 ppt, 0.05 ppt, 0.08 ppt, and 0.11 ppt malathion was added to 999 ml of 30‰ filtered seawater daily to give the final concentrations of 0.02 ppm and 0.11 ppm.

Fresh stock solutions of malathion were shipped to Beaufort, N. C., by Hazleton Laboratories before each experiment with larvae of one of the species of crabs. The stock solutions were stored in a coldroom at 5°C at the Duke University Marine Laboratory to prevent deterioration during the course of an experiment.

Source of mother crabs and hatching of eggs

Ovigerous Rhithropanopeus harrisii Gould, small mud-crabs belonging to the family Xanthidae, were collected from the west side of Newfound Harbor, a small estuary which is between the Indian River to the west and the Banana River to the east in the vicinity of Cocoa Beach, Florida. They were shipped to North Carolina from Florida by air freight in January and February 1974 and 1975. Upon arrival in Beaufort, N. C., each small mud-crab was placed in a large glass finger bowl (19.4 cm diam.) containing filtered seawater with a salinity of 20‰, the salinity to be used during rearing of larvae for experiments with methoxychlor and malathion. The ovigerous crabs were maintained in a culture cabinet at 25°C and with a light regime of 12 hours light and 12 hours darkness.

Ovigerous Callinectes sapidus Rathbun, commercial blue crabs belonging to the family Portunidae, were collected from the Beaufort Inlet, N.C. They furnished larvae for experiments with mirex in 1973 and with methoxychlor and malathion in 1974. The crabs used carried black eggs which had eyes and visible heartbeat. The method for developing the eggs was the same as Costlow and Bookhout described in 1960. Pleopods bearing eggs were removed, placed in large glass finger bowls (19.4 cm diam.) of filtered seawater at 30‰, the salinity to be used for rearing larvae. Setae bearing eggs were removed from pleopods with fine scissors, and washed 3-5 times in filtered seawater.

The eggs were further dissociated with glass needles and transferred to freshly filtered seawater. They were washed in an additional bowl of filtered seawater and then placed in plastic compartmented boxes, 32.5 cm x 22.7 cm, or 2000 ml flasks of filtered 30‰ seawater. These containers were then placed on an Eberbach variable speed shaker regulated for 60 oscillations min^{-1}. Black eggs with eyes and a visible heartbeat usually hatched within one or two days after they were placed on the shaker.

Rearing of larvae in check series

The methods of rearing larvae of Rhithropanopeus harrisii and Callinectes sapidus in acetone control and 4 to 5 concentrations of the insecticides mirex, methoxychlor and malathion were essentially the same for the two species. Larvae of R. harrisii were reared in a salinity of 20‰ and at a temperature of 25°C, because this was the salinity and temperature found to be optimum in earlier studies (Costlow, Bookhout and Monroe, 1966). They were reared on Artemia salina nauplii. Callinectes sapidus larvae were reared at a salinity of 30‰ and a temperature of 25°C, the salinity and temperature found to be optimum in rearing studies at the Duke University Marine Laboratory from 1959 (Costlow and Bookhout, 1959) to the present. Callinectes sapidus larvae were fed Artemia salina nauplii and fertilized Arbacia eggs.

As soon as the eggs from one mother crab hatched, 10 larvae were placed in each of the finger bowls (8.9 cm in diameter) to be used in the series. The series included a given number of bowls of control larvae in 1 ppt acetone in filtered seawater, and the same number of bowls of larvae in 4 or 5 concentrations of the insecticide. A drop of *Artemia salina* nauplii, hatched from California eggs, and a drop of fertilized *Arbacia* eggs were added to each bowl containing *C. sapidus* larvae, but only *Artemia salina* nauplii were fed to *R. harrisii* larvae. On the following day and each day thereafter, the number of living larvae, and the number and stages of dead larvae in each bowl were recorded. Hence, this series is referred to as a check series. Living larvae were transferred to clean bowls with fresh media, and food was added daily. As each zoea molted to a megalopa, it was transferred to a separate division of a compartmented box containing 25 ml of the desired medium. The liquid contents were removed from each compartment and fresh medium and food were added daily until a megalopa molted to the 1st crab stage. Larvae from each replicate mother crab were handled in the same manner. Thus each mother crab furnished enough larvae to be reared in acetone control and the concentrations of the insecticide for each replicate.

Rearing of larvae for residue analysis

Mass cultures of *R. harrisii* and *C. sapidus* were maintained for residue analyses in large glass finger bowls (19.4 cm diam.) containing the same media as the check series. Initially approximately 1000 freshly hatched larvae were placed in a bowl containing 700 ml of one of the media. A medicine dropper full of *Artemia* nauplii and the same volume of fertilized *Arbacia* eggs were added to each bowl, if the larvae were *C. sapidus*, but were fed *Artemia* nauplii only if the larvae were *R. harrisii*. Living larvae were transferred to a clean bowl and fed daily. To obtain 0.20 to 0.25 grams of larvae (wet weight) for residue analyses, 10 large bowls of larvae in acetone control and 10 to 16 bowls in each of the concentrations of the insecticide were maintained. Mortality of larvae was much greater in mass cultures than in check series, hence in the higher concentration of insecticides, it was not possible to obtain enough larvae in late stages to analyze. If larvae survived to the 1st crab stages in check series, these were obtained for analysis.

Larvae collected for analysis were washed seven times in filtered seawater, blotted on filter paper, weighed, wrapped in aluminum foil and stored frozen. Wrapped samples in aluminum foil were shipped in dry ice to Hazleton Laboratories for analysis.

Residue analyses

Determination of chlorinated pesticide residues in mother crabs, *Rhithropanopeus harrisii* and *Callinectes sapidus*, was made by Hazleton Laboratories, Inc., in 1973, and by the same laboratory but known as Hazleton Laboratories America, Inc., in 1974 and 1975. This company also made mirex determinations of *C. sapidus* mother crabs and their larvae in 1973 and methoxychlor determinations in larvae of both species in 1974 and 1975. Their methods are described below.

Weighed samples were ground with 15 g anhydrous sodium sulfate to absorb the moisture and transferred with petroleum ether rinsings to a 500-ml round bottom flask. The sodium sulfate and crabs were extracted three times with 10-ml petroleum ether and filtered through 10 g sodium sulfate retained on a fritted glass funnel into a 250-ml round bottom flask. The extract was transferred with petroleum ether rinsings into a 125-ml separatory funnel and extracted four times with 25-ml acetonitrile saturated with petroleum ether. Each 25-ml was transferred to a 500-ml separatory funnel containing 300-ml of 2% sodium chloride and 100-ml petroleum ether. After combining all extracts in the 500-ml separatory funnel, it was shaken gently for 1 minute to transfer the pesticides to the petroleum ether. The lower aqueous layer was discarded and the petroleum ether washed twice more with 50-ml 2% sodium chloride. After discarding the washings, the lower petroleum ether layer was drawn off into a glass stoppered bottle containing 10 g sodium sulfate.

The extract was transferred to an activated (5 hours at 130° C) florisil (pesticide grade floradin) column (I.D. 22 mm) containing 100-mm after packing and topped with about ½ inch of anhydrous sodium sulfate. The petroleum ether extract was passed through the column at a rate of 3 ml/minute. When the last of the extract had sunk into the column the pesticides (BHC, Lindane, Chlordane, Aldrin, Heptachlor, Heptachlor Epoxide, DDE, DDD, DDT, Aroclor 1242, Aroclor 1254, HCB, and Methoxychlor) were eluted with 110-ml of 6% ethyl ether in petroleum ether at the same rate. After the last of the 6% ethyl ether fraction had sunk into the sodium sulfate layer the receptacle was changed and the column was eluted with 200-ml of 50% ethyl ether in petroleum ether at the same rate. This fraction elutes the remaining pesticides (Endrin and Dieldrin). Both elutes were then concentrated to 2-ml on a flash evaporator (water bath, 40°C). The concentrated extracts were transferred with rinsings to 15-ml centrifuge tubes and concentrated further, if necessary, using a stream of dry nitrogen and warm water bath.

Gas Chromatography

The concentrated samples were analyzed by gas chromatography using an electron capture detector. The gas chromatograph was operated using the following parameters:

Apparatus:	Micro-Tek, MT-220
Detector Type:	Electron Capture, Ni^{63}, RF Pulsed
Recorder Ranger:	1 mv Full Scale, Chart Speed 1/3 inch per minute
Column:	6' x ¼" O.D., Glass, 3% OV 17 on 100/120 mesh, Gas Chrom-Q
Temperatures:	
Injection Port -	270°C
Detector -	280°C
Column Oven -	225°Isothermal
Gas Flow:	Nitrogen, 5.5 on Flow Meter and 40 psi at the Tank
Attenuation:	10 x 2

Dr. Richard Stanovick, Director of the Biochemistry Division, Hazleton Laboratories America, Inc., stated that the expected recovery of residues in the analyses for mirex would be at the level of 82%, for methoxychlor at the level of 90% and for the analyses of pesticides in ovigerous crabs between 80-90%.

Studies related to sexual morphogenesis

To provide material to determine sexual variations due to methoxychlor and malathion, *Rhithropanopeus harrisii* and *Callinectes sapidus* were reared from the last zoeal stage to the 6th crab stage in acetone control and in the same concentrations of methoxychlor and malathion as described previously. For Dr. Genevieve Payen, University of Paris, to conduct a study of sexual morphogenesis, we fixed in alcoholic Bouin's (Dubosq-Brasil) 15 megalopa and up to 15 of each of the 6 crab stages which had been reared in each of the concentrations of methoxychlor and malathion. The sexual variants which received particular attention were the pleopods, abdominal appendages, and the primary sexual characters, such as the gonads, their ducts and the androgenic gland.

The sequence of observations started with larvae which had been reared in the highest concentration and continued with larvae reared in lower concentrations of the insecticide until no modification of pleopods could be detected. Such larvae were found in the following concentrations: 7.0 ppb, 5.5 ppb and 4.0 ppb methoxychlor for R. harrisii; 1.9 ppb and 1.6 ppb methoxychlor for C. sapidus; 0.02 ppm and 0.017 ppm malathion for R. harrisii and 0.11 ppm and 0.08 ppm malathion for C. sapidus.

Pleopods of control and contaminated crabs were dissected either from the intact or isolated abdomen. The number and character of setae and any morphological abnormality of the pleopods were recorded. Whole mounts were stained with fast gree or safran, and lactic acid was used to increase the transparency of the tissues.

In order to determine the extent of development of the gonads, its ducts and the androgenic glands, it was necessary to make parasagittal and transverse sections. These were cut at 5µ and stained with picroindigo-carmin and hematoxylin. Serial sections were examined to determine if the genital apparatus was developing normally or abnormally compared to the controls and to reference slides of each species made by Dr. Payen before this project started.

SECTION V

MIREX

INTRODUCTION

Mirex, a chlorinated hydrocarbon, is the active ingredient in bait used to control the imported red ant, Solenopsis invecta Buren, in the Southeastern United States, and Solenopsis richteri in restricted areas in northeastern Mississippi and northern Alabama (Alley, 1973). The bait, first used by the U.S. Department of Agriculture (USDA) is designated as 4X bait. It consists of 84.7% corn-cob grits, 15% soybean oil, and 0.3% mirex. It was applied at a rate of 1.4 kg/ha (kilograms per hectare); (1.25 pounds per acre) (Coon and Fleet, 1970). Within recent years 2X bait with 0.15% mirex, and 1X bait with 0.1% mirex has been used (Alley, 1973).

Lowe et al. (1971) found 4X mirex bait and/or technical mirex to be toxic to decapod crustaceans in the laboratory and in simulated field conditions. Juvenile brown shrimp, Penaeus aztecus, grass shrimp, Palaemonetes pugio, juvenile blue crabs, Callinectes sapidus, and fiddler crabs, Uca pugilator, were poisoned. Although adult and subadult blue crabs (76 to 127 mm) were not affected by ten times the USDA's suggested application rate of 1.4 kg/ha, McKenzie (1970), and Lowe et al. (1971) found that juvenile blue crabs are more sensitive to mirex bait, the smaller they are.

Crayfish, Procambarus blandingi, are sensitive to technical mirex concentrations from 0.1 ppb to 5.0 ppb, to 4X bait, and also to mirex which leaches out of 0.3% bait (Ludke et al., 1971). Redmann (1973) reported that grass shrimp, Palaemonetes pugio, are poisoned by concentrations of 0.01 ppm to 1.0 ppm technical mirex, to 0.15% mirex bait, and to mirex which leached out of the bait.

At a time when mirex was being aerially applied to coastal areas from North Carolina to Florida, Mahood et al. (1970) found mirex residues to be the second most common insecticide in monthly samples of blue crabs. The percent of blue crabs with residues of mirex was directly proportional to the amount of spraying.

Borthwick et al. (1973) monitored the movement and accumulation of mirex in selected estuaries from 1969-71, when mirex was aerially applied on October, 1969, June and October, 1970, to coastal areas to eradicate fire ants near Charleston, S.C. Mirex was not detected in any pretreatment samples of crabs, shrimp, fish, water or sediment. After treatment, the greatest number of organisms with mirex in estuaries were taken at stations located closest to treated areas. Organisms downstream from the treated areas also accumulated mirex, but in lesser amounts. Residues of mirex were more often found in crabs than in shrimp or fish. The authors were of the opinion that mirex moved from treated land to water after each treatment. They did not explain the mechanisms involved, but they suspected surface runoff after heavy rainfall was a likely method. Naqvi and de la Cruz (1973) made a similar study of accumulation of mirex by freshwater aquatic organisms near treated areas in Mississippi, and reported widespread movement of mirex in the environment. They were of the opinion that mirex residues in terrestrial organisms were the result of animals consuming mirex bait, whereas mirex residues in aquatic organisms were obtained from leaching of the pesticide from bait.

Since it has been shown that juvenile blue crabs are more sensitive to mirex, the smaller they are, it might be suspected that larvae in developmental stages of Callinectes sapidus might be even more sensitive than juveniles. Indirect evidence for this suspicion was given by Buchanan et al. (1970), for he determined that early larvae of the dungeness crab, Cancer magister, were more sensitive to Sevin, a carbamate pesticide, than juveniles, and juveniles were more susceptible than adults. There was no direct evidence to support or disprove this suspicion in the case of blue crab larvae, however, until the portion of this report dealing with mirex was published (Bookhout and Costlow, 1975). Only one other paper had appeared on the effect of mirex on the complete development of crabs. It concerned the chronic effects of mirex on the mud-crab, Rhithropanopeus harrisii, and the stone crab, Menippe mercenaria (Bookhout et al., 1972). The objectives of the current study were to determine the effects of technical mirex on the complete larval development of Callinectes sapidus from the time of hatching to the 1st crab stage. Specific answers to the following questions were sought: (1) what concentrations of mirex are sublethal and which are acutely toxic;

(2) what are the effects of sublethal concentrations; (3) are larvae in one or more developmental stages particularly sensitive to different concentrations of mirex; (4) what residues of mirex are present in larvae of different ages in reference to concentration and length of exposure; and, (5) what are the differences in the effects of mirex on the complete development of C. sapidus, R. harrisii and M. mercenaria?

This section is a final report of what has been done and published (Bookhout and Costlow, 1975) on the effect of mirex on the complete development of the blue crab, Callinectes sapidus.

RESEARCH RESULTS

EFFECTS OF MIREX ON DEVELOPMENT OF Callinectes sapidus

Survival

Records of day to day percent survival of 200 Callinectes sapidus larvae from mother crab, Cs II, indicate that there was no appreciable difference in survival of larvae reared in acetone control and in 0.01 ppb, 0.1 ppb, 1.0 ppb and 10.0 ppb mirex during the first five days. Thereafter, there was a sharp reduction in survival of larvae reared in 10.0 ppb mirex until there were none living by day 13. Larvae reared in 1.0 ppb mirex showed a sharp reduction in survival from 95% to 35% between the days of 8 and 15. From day 15 to 58 there was a more gradual reduction until only one (0.5%) 1st crab out of 200 of the original number of larvae was left. Larvae reared in acetone control, 0.01 ppb and 0.1 ppb mirex showed differential survival with the highest percent surviving to megalopa and to 1st crab stage in acetone control and the lowest in 0.1 ppb (Table 1).

One hundred larvae hatched from mother crab, Cs VI, were also reared in acetone control and in the same concentrations of mirex as larvae from Cs II. Again there was no appreciable difference in survival of larvae reared in any of the media for the first 5 days. Larvae reared in 10.0 ppb mirex only survived to 12 days and those maintained in 1.0 ppb mirex lived to 20 days. Percent survival to megalopa and to 1st crab stage is given in Table 1.

The average percent survival of 300 larvae from Cs II and Cs VI reared in five media is plotted in Figure 1.

TABLE 1

EFFECT OF MIREX ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Callinectes sapidus* II and VI

Culture Media Salinity 30‰ Temp. 25°C	Initial # of Larvae per Series	Mean Duration of			% Survival to	
		Zoeal Development in Days	Megalopa Development in Days	Hatching to 1st Crab in Days	Megalopa	1st Crab
Acetone Control	Cs II 200	32.28	8.55	41.21	60.5	52.0
Mirex 0.01 ppb	Cs II 200	32.90	8.76	41.60	56.0	41.5
Mirex 0.1 ppb	Cs II 200	31.06	8.83	41.00	50.5	30.5
Mirex 1.0 ppb	Cs II 200	42.50	7.00 (one)	51.00 (one)	2.0	0.5
Mirex 10.0 ppb	Cs II 200					
Acetone Control	Cs VI 100	37.75	11.16	48.75	60	55
Mirex 0.01 ppb	Cs VI 100	38.07	11.73	49.55	44	40
Mirex 0.1 ppb	Cs VI 100	38.49	10.63	49.13	41	30
Mirex 1.0 ppb	Cs VI 100					
Mirex 10.0 ppb	Cs VI 100					

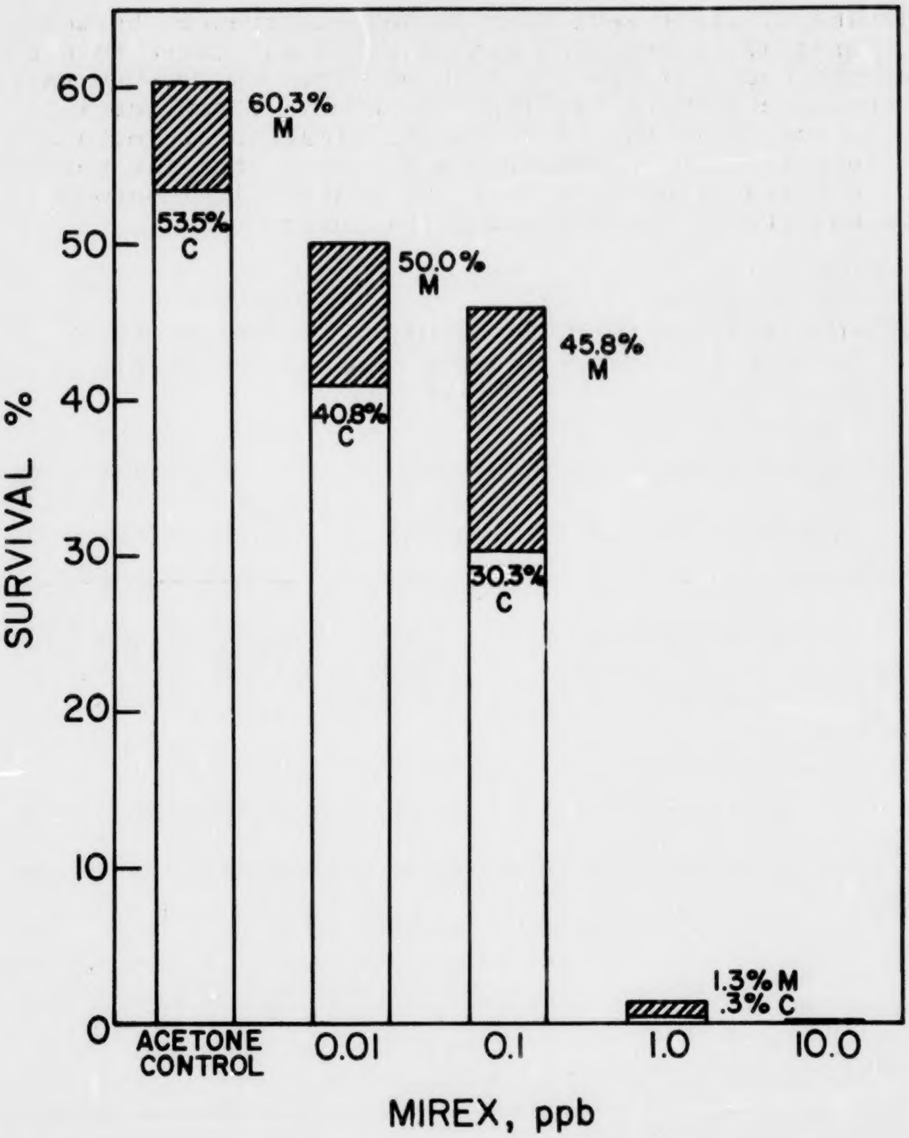

Figure 1. Average percent survival of two replicate series of *Callinectes sapidus* larvae, Cs II and Cs VI, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of mirex.

To determine if there were significant differences between percent survival to megalopa and to 1st crab stages in acetone control and 0.01 ppb and 0.1 ppb mirex, a two-way analysis of variance (ANOVA) was made. There were replicate larvae from crabs Cs II and Cs VI per combination of media and stage (Table 1). The ANOVA (Table 2) shows that the percent survival differs significantly (0.01 probability) between megalopa and crab stage and among the three media.

TABLE 2

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MIREX ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* II and VI

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	5	1095.25	219.05	
A (Stages)	1	330.75	330.75	16.13 **
B (Media)	2	723.88	361.94	17.66 **
A x B (Interaction)	2	40.63	20.31	0.99 n.s.
Within subgroups (error)	6	123.00	20.50	
Total	11	1218.25		

Significant F ratios: F .01 [1,6] = 13.75, F .01 [2,6] = 10.93

Since the interaction between stage and media is clearly nonsignificant (Table 2), the results may be summarized by the stage difference and the media effect (Table 3). The survival to megalopa is 10.5% ± 2.6% greater than that to crab stage. The media effect may be expressed as a decrease of 9.4% ± 1.6% in survival for each 10-fold increase in mirex concentration in the range studied.

TABLE 3

MEAN PERCENT SURVIVAL TO MEGALOPA AND TO FIRST CRAB IN *C. sapidus* II and VI

Media	Survival to Megalopa	Survival to 1st Crab	Average
Acetone control	60.3	53.5	56.9
0.01 ppb	50.0	40.8	45.4
0.1 ppb	45.8	30.3	38.0
Average	52.0	41.5	

Duration of Development

Larvae hatched from mother crab, Cs II, on May 1, 1973, and reared in acetone control, 0.01 ppb and 0.1 ppb mirex showed remarkable consistency in mean duration of zoeal development, megalopa development and in total time from hatching to 1st crab stage (Table 1). Larvae hatched from mother crab, Cs VI, on June 18, 1973, and reared in the same media, took a longer time to complete zoeal and megalopa development than larvae from Cs II, but the time span of larvae reared in each of the three media was similar (Table 1). Larvae of blue crabs reared later in the breeding season showed a longer period of development than those reported here. Thus the increased time of development of larvae from Cs VI may be related to time in the breeding season the larvae hatched, rather than to the media in which they were reared. Larvae from each crab which were reared in 1.0 ppb and 10.0 ppb mirex were slower to molt than larvae reared from each crab in 0.01 ppb and 1.0 ppb. This is considered a sublethal effect of mirex. All of these larvae died with the exception of 2% of larvae from Cs II which completed zoeal development in 1.0 ppb mirex in 42.50 days compared to 31 to 32 days in other media (Table 1).

Mortality

A record of deaths in each of the eight zoeal stages and megalopa was made for larvae reared from mother crabs, Cs II and Cs VI (Table 4), in an effort to determine if larvae in one or more stages of development were particularly sensitive to different concentrations of mirex.

A single classification ANOVA of the mortality data in Table 4, using replicates from larvae of mother crabs Cs II and Cs VI, indicates that larvae in no one stage were significantly sensitive statistically to 0.01 ppb. On the other hand, larvae in zoeal stages III, VII and megalopa in 0.1 ppb mirex, and larvae in zoeal stages II and III in 1.0 ppb. showed significantly greater mortality than other stages. In 10.0 ppb mirex, all larvae died in zoeal stage I and II.

TABLE 4

PERCENT MORALITY IN NINE DEVELOPMENTAL STAGES
OF *C. sapidus* II and VI

Stage	Control		Mirex							
	Acetone		0.01 ppb		0.1 ppb		1.0 ppb		10.0 ppb	
	Cs II	Cs VI	Cs II	Cs VI	Cs II	Cs VI	Cs II	Cs VI	Cs II	Cs VI
I	1.0	3	0.5	7	0.0	2	6.0	9	53.5	95
II	0.5	11	2.0	28	2.5	7	34.0	78	46.5	5
III	7.5	7	10.5	7	16.5	13	24.5	13	-	-
IV	0.5	4	3.5	2	1.5	4	10.0	-	-	-
V	0.5	1	0.0	1	0.0	2	2.0	-	-	-
VI	0.5	4	1.0	0	0.5	4	1.0	-	-	-
VII	24.0	9	26.0	10	28.5	20	13.0	-	-	-
VIII	5.0	1	0.5	1	0.0	7	3.5	-	-	-
Megalopa	8.5	5	14.5	4	20.0	11	1.5	-	-	-
Total	48.0	45	58.5	60	69.5	70	99.5	100	100.0	100

Residue Analysis

Larvae of three mother crabs Cs II, Cs III and Cs V were reared in mass cultures in acetone control, 0.01 ppb, 0.1 ppb, 1.0 ppb and 10.0 ppb mirex to obtain enough wet weight of larvae (0.30 to 0.20 g) for residue analysis. These three crabs plus mother crab Cs VI and Cs VII were analyzed by the Hazleton Laboratories for residues of the following pesticides: BHC, Lindane, Chlordane, Aldrin, Heptachlor, Heptachlor Epoxide, DDE, DDD, DDT, Aroclor 1242, Aroclor

1254, Endrin, Dieldrin and HCB. None of the pesticides were found in detectable amounts, <0.01 ppm, with the exception of DDE, DDD and DDT. The latter are given in Table 5.

TABLE 5

RESIDUES OF DDE, DDD AND DDT IN FIVE MOTHER CRABS

Sample	DDE (ppm)	DDD (ppm)	DDT (ppm)
Cs II	0.03	0.02	0.07
Cs III	0.03	<0.01	0.06
Cs V	0.03	0.04	0.03
Cs VI	0.02	<0.01	0.01
Cs VII	0.01	<0.01	0.01

Residue analyses in larvae were also made (Table 6) to obtain information concerning the relationship between length of time in each concentration and residues of mirex, and between increase in residues and increase in concentration. No detectable mirex, <5 ppb, was found in freshly hatched larvae in seawater, or in 5 and 15 day larvae, megalopa, or in 1st and 2nd crabs which had been reared in acetone control. Larvae reared in a range of concentrations of mirex from 0.01 ppb to 10.0 ppb showed increased residues of mirex with increase in concentration (Table 6).

According to the residue analyses, the biological magnification of mirex was greatest in larvae reared in 0.01 ppb and decreased with concentration thereafter.

Mother crabs whose larvae were used to obtain enough larvae for residue analysis had no detectable mirex, <5 ppb, except Cs II which had 10.0 ppb.

DISCUSSION

Effect of Mirex on Survival

Mirex, in a range from 0.01 ppb to 10.0 ppb, had no appreciable effect on day to day survival of two replicate series of blue crab larvae for five days after hatching. Delayed mortality was also noted in the early stages of zoeal development of *Rhithropanopeus harrisii* and *Menippe mercenaria*

TABLE 6

MIREX RESIDUES IN BLUE CRABS AND THEIR LARVAE

Sample Identification and Treatment	ppb Mirex
First day larvae - Seawater	<5
5 day larvae - Acetone Control	<5
15 day larvae - Acetone Control	<5
Megalopa - Acetone Control	<5
Crabs, 1st & 2nd - Acetone Control	<5
5 day larvae - 0.01 ppb Mirex	11
15 day larvae - 0.01 ppb Mirex	30
Megalopa - 0.01 ppb Mirex	20
Crabs, 1st & 2nd - 0.01 ppb Mirex	9
5 day larvae - 0.10 ppb Mirex	33
15 day larvae - 0.10 ppb Mirex	70
Megalopa - 0.10 ppb Mirex	65
Crabs, 1st & 2nd - 0.10 ppb Mirex	77
5 day larvae - 1.0 ppb Mirex	301
8 day larvae - 1.0 ppb Mirex	406
5 day larvae - 10.0 ppb Mirex	1620
8 day larvae - 10.0 ppb Mirex	1370
Mother Crab Cs II - Seawater	10
Mother Crab Cs III - Seawater	<5
Mother Crab Cs V - Seawater	<5

reared in the same concentrations of mirex (Bookhout *et al*., 1972). The only significant mortality of first stage zoeae was observed in *M. mercenaria* reared in 10.0 ppb. Delayed toxicity of mirex to juvenile blue crabs has also been reported by MacKenzie (1970); by Lowe *et al*. (1971) to juvenile pink shrimp, *Penaeus duorarum*; by Ludke *et al*. (1971) to two species of crayfish, *Procambarus blandingi* and *P. hayi*; and by Plapp (1973) to house flies, *Musca domestica*. MacFarlane *et al*. (1975) noted that the characteristic feature of mirex poisoning to the field cricket, *Gryllus pennsylvanicus*, was the long latent period of 72 hours after it was treated with a lethal dose of mirex. During this period the activity of the ventral nerve cord was the same as untreated individuals and the behavior of treated crickets was the same as untreated individuals. The long latent period before poisoning of the nerve cord may also account for the delayed toxicity to adult and larval crustaceans. Investigators do not often define what they mean by sublethal and lethal concentrations of an insecticide in chronic bioassay studies of decapods. In a chronic bioassay investigation of the effect of dieldrin on the development of two crabs, Epifanio (1971) considered sublethal concentrations as those in which there is differential survival with increased concentration of the toxicant in relation to survival in the control medium and those in which more than 10% of the larvae reach the 1st crab stage. Acutely toxic concentrations are those in which 10% or less of the larvae reach the 1st crab stage. These definitions, in our opinion, are valid when survival to the 1st crab stage is high (above 30%) in the control, but not when survival is low (below 30%).

After a five day period of delayed mortality in all experimental concentrations of mirex, two concentrations were shown to be sublethal and two acutely toxic to blue crab larvae. Since 41.5% to 30% of two replicate series of larvae of blue crabs reached the 1st crab stage when reared in 0.01 ppb and 0.1 ppb mirex, these concentrations are considered to be sublethal. These concentrations of mirex are also sublethal to *Rhithropanopeus harrisii* and *Menippe mercenaria* (Bookhout *et al.*, 1972). Sixty-seven percent and 49% of *R. harrisii* larvae reached the 1st crab stage compared to 9% and 5% of *M. mercenaria* larvae. In the control, survival to the 1st crab stage in *R. harrisii* was 88% and 27% in *M. mercenaria*. Concentrations of 1.0 ppb and 10.0 ppb proved to be acutely toxic in the three species of crabs which have been reared in mirex. In one series of blue crab larvae 0.5% out of 200 larvae reached the crab stage in 1.0 ppb, but no larvae from the second series survived beyond 20 days. In *R. harrisii*, 7% reached the crab stage in 1.0 ppb, and no larvae survived beyond the megalopa in 10.0 ppb mirex. In *M. mercenaria*, no larvae developed beyond the megalopa stage in 1.0 ppb, or beyond the 3rd zoeal stage in 10.0 ppb mirex (Bookhout *et al.*, 1972).

Juvenile blue crabs showed no symptoms of poisoning during a 96 h exposure to 0.1 ppm technical mirex in flowing seawater, but died within 18 days in mirex-free seawater (Lowe *et al.*, 1970). Tagatz *et al.* (in press) found mirex in concentrations of 0.04 ppb to 0.12 ppb which had leached from mirex bait to be lethal to juvenile blue crabs within 6 to 28 days of exposure. Although these experiments are different from the chronic study of blue crab larvae, it appears that Callinectes sapidus larvae are about as sensitive to mirex as small juvenile blue crabs.

Larvae of C. sapidus, R. harrisii and M. mercenaria seem to be as sensitive to mirex as juvenile crayfish and juvenile pink shrimp. Sixty-five percent and 71% of the juvenile crayfish, Procambarus blandingi and P. hayi, died after being exposed to 0.1 ppb and 0.5 ppb mirex for 48 h (Ludke et al., 1971). Eleven percent of 36 juvenile pink shrimp, Penaeus duorarum, died during 3 weeks exposure to 0.1 ppb technical mirex in flowing seawater and 25% died in mirex-free water during a two-weeks post-treatment period (Lowe et al., 1971).

Adult grass shrimp, Palaemonetes pugio, seem to be intermediate in sensitivity to mirex between juvenile blue crabs and blue crab larvae. After adult grass shrimp were exposed to 0.01 ppm mirex for 48 h, 40% died after 12 days. When they were exposed to 0.1 ppm and 1.0 ppm for 48 h, 91% died in 0.1 ppm after 10 days, and 100% died in 1.0 ppb after 5 days (Redmann, 1973).

Sublethal Effects

The duration of zoeal development and the time from hatching to the 2nd crab stage of Rhithropanopeus harrisii increased significantly with increase in concentration of mirex from 0.01 to 10.0 ppb (Bookhout et al., 1972). The reduction in molting rate is considered a sublethal effect of mirex. In the two series of Callinectes sapidus larvae from mother crabs, Cs II and Cs VI, the duration from hatching to megalopa and to 1st crab reared in acetone control and in 0.01 ppb and 0.1 ppb showed no statistical difference in duration. In the acutely toxic concentration of 1.0 ppb mirex only 2% of the larvae of Cs II reached the megalopa stage, but it took them 11 days longer to do so than those reared in sublethal concentrations of 0.01 ppb and 0.1 ppb mirex (Table 1). Larvae in 1.0 ppb and 10.0 mirex showed a slower rate of molting compared to larvae reared in acetone control and 0.01 ppb and 0.1 ppb mirex, hence this trend is considered a sublethal effect of mirex.

In Menippe mercenaria, there were no significant differences in duration of developmental stages or time to reach the megalopa stage in relation to concentration of mirex, but extra 6th zoeal stages increased from 65% to 66% to 90% in concentrations of 0.01 ppb, 0.1 ppb and 1.0 ppb mirex, respectively, compared to 2.5% in seawater control. This is another type of sublethal effect of mirex. The majority of the 6th zoeal stages died before reaching the megalopa stage (Bookhout *et al*., 1972). Blue crabs normally pass through seven zoeal stages, and occasionally eight zoeal stages, before molting into a megalopa stage (Costlow and Bookhout, 1959). Based on the tabulation of mortality of the 8th zoeal stages in acetone control and in concentrations of mirex from 0.01 ppb to 1.0 ppb (Table 4), there is no evidence that extra 8th zoeal stages increased with higher concentrations of mirex. The majority of 8th zoeal stages of *C. sapidus*, like extra 6th stages of *M. mercenaria*, usually died before molting into megalopa.

Residues

Table 6 shows that residues of mirex increased with concentration from 0.01 ppb to 10.0 ppb. In the sublethal concentrations of 0.01 ppb and 0.1 ppb mirex, residues increased in 5 to 15 days from 11 ppb to 30 ppb, and 33 ppb to 77 ppb, respectively. Larvae reared in 0.01 ppb mirex had a residue of 20 ppb in the megalopa and 9 ppb in the crab stage, but larvae reared in 0.1 ppb had residues of 65 ppb in the megalopa and 70 ppb in the crab stage, or about the same as the residue in larvae at 15 days. Ludke *et al*. (1971) also noted that residues in crayfish, which had been exposed to two granules of mirex bait, increased initially, but remained fairly constant after the second day.

In the acutely toxic concentration of 1.0 ppb mirex, residues were 301 ppb at 5 days and 406 ppb at 8 days, whereas in the lethal concentration of 10.0 ppb, residues were 1620 ppb at 5 days and 1370 ppb at 8 days. At 8 days, larvae in these concentrations were dying in large culture bowls. Hence, it was not possible to obtain enough larvae for residue analysis at 15 days.

Biological magnification of mirex during larval development of blue crabs was greatest in larvae reared in 0.01 ppb and decreased with concentration from 0.1 ppb to 10.0 ppb mirex. When Jernelöv *et al*. (1972) examined the accumulation of components 1-6 of the EDC-tar, a mixture of short chained aliphatic hydrocarbons, in the shrimp, *Leander adspersus*, and the mussel, *Mytilus edulis*, they too found that accumulation was highest when EDC-concentration was lowest and lowest when EDC-concentration was highest.

After 54 hours of exposure to 0.86 ppb mirex which had leached from mirex bait, 33 of 35 juvenile crayfish died.

These were divided into three samples for analysis. They had body residues of 1.602, 1.409, and 1.339 ppm mirex (Ludke *et al.*, 1971). These residues are comparable to those found in blue crab larvae at five and eight days which had been reared in 10.0 ppb.

Juvenile shrimp, *Penaeus duorarum*, exposed to 0.1 ppb of technical mirex for three weeks and analyzed for residues two weeks after being in mirex-free seawater contained 0.32 ppm (Lowe *et al.*, 1971) which is comparable to residues detected in blue crab larvae reared in 1.0 ppb mirex at five days.

Three juvenile blue crabs, which were fed a fish a day for five days that contained 1.0 ppm mirex and uncontaminated fish for three weeks thereafter, exhibited symptoms of mirex poisoning or were dead (Lowe *et al.*, 1971). These crabs had residues of 0.13 ppm, 0.22 ppm and 0.25 ppm mirex. These values are slightly less, but comparable to residues of mirex found in blue crab larvae reared in 1.0 ppb at five and eight days.

Pooled samples of Crustacea, collected from lakes, ponds, creeks and an estuary in areas which had varying degrees of mirex treatment, had mirex residues which averaged 0.44 ppm (Naqvi and de la Cruz, 1973). This value is very close to the residue of mirex found in blue crab larvae reared in 1.0 ppb at eight days.

From the examples given, it appears that juvenile *Procambarus blandingi* and juvenile *Penaeus duorarum* concentrate leached or technical mirex, presumably through the gills, more efficiently than blue crab larvae, since they reached approximately the same residue level when reared in 1/10 less concentrated medium of mirex. When mirex is obtained through food, as in the cited case of juvenile blue crabs, accumulation is slower than when mirex is obtained from technical mirex. Epifanio (1973) reported that *Leptodius floridanus* larvae accumulated dieldrin 19.1 times as fast from 0.5 ppb in seawater as from 213 ppb in food. This, he believed, is because the pumping rate is much higher than the feeding rate.

SECTION VI

METHOXYCHLOR

INTRODUCTION

Methoxychlor is one of the chlorinated hydrocarbon insecticides which is used to replace DDT, now banned in the United States. It is employed chiefly to control blackfly larvae in streams, the smaller European elm bark beetle, and a variety of pests on fruits, vegetables and forage (Burdick *et al.*, 1968; Wallner *et al.*, 1969; U.S. Department of Agriculture, 1968). As a substitute chemical for DDT, methoxychlor is nearly as toxic to the target organism, yet biodegradable. Hence, it does not give rise to such long lasting residues as DDT (Burdick *et al.*, 1968; Metcalf *et al.*, 1971).

Methoxychlor is stable to heat and resistant to oxidation and relatively stable to ultraviolet radiation. Henderson *et al.* (1959) concluded that pH and alkalinity has no major effect on the toxicity of methoxychlor to fish, but found that the insecticide was somewhat more toxic to fish in hard water, 400 ppm, than in soft water, 20 ppm. Merna *et al.* (1972) reported that hydrogen ion concentration in distilled water within a range of pH 7 to 9 had no effect on the breakdown rate of methoxychlor.

Several investigators have been concerned with the breakdown of methoxychlor and the accumulation of methoxychlor residues in the environment. Burdick *et al.* (1968) exposed brook trout to 5 μg/l (micrograms per liter-parts per billion) methoxychlor for two day periods a week apart. At the end of one week in clean water, the trout lost 41.3% of the residue they gained during the two days of exposure. After three months no residue was detected. Wallner *et al.* (1969) reported that after American elms were sprayed with methoxychlor from a helicopter, substantial residues were found in twig crotches and soil beneath the trees for one year after spraying. Water samples from a stream adjacent to the treated trees showed most residues in the top inch, but the residues were diluted by the current of the stream and were undetected after 24 hours. Samples taken later at two week intervals from spring to fall showed 1 part per trillion methoxychlor in water and 1.0 ppm in silt.

Using freshwater mussels, *Lampsilis siliquoidea* and *L. ventricosa*, as monitors, Bedford *et al*. (1968) found that the mussels concentrated methoxychlor from river water adjacent to an area where there had been a spraying for Dutch elm disease. The concentrations at two stations were 0.1190 ppm and 0.0702 ppm after two weeks, increased to a high of 0.1310 ppm and 0.2221 ppm after six weeks and returned after ten weeks to a level found after two weeks. These results indicate that methoxychlor may remain in water for a longer time than Wallner *et al*. (1969) reported.

The retention of methoxychlor in silt may be associated with biomagnification of the insecticide by bacteria. Johnson and Kennedy (1973) reported that *Aerobacter aerogenes* and *Baccillus subtilis* accumulated ^{14}C-labeled methoxychlor directly from water. The uptake by both species was rapid; 80 to 90% of the 24-h residues occurred within 30 min. In water ranging from 0.5 to 50 μg/liter, the residue magnification factors from the water were between 1,400 to 4,300 fold. If bacteria can accumulate methoxychlor and concentrate it over one thousand times above ambient water levels, they could be the means of transfer of the insecticide to higher levels of the food chain for they serve as a nutrient source for filter feeding organisms.

Although methoxychlor is considered a moderately hazardous insecticide, it may be highly toxic to some aquatic organisms. When juvenile and adult striped mullet, *Mugil cephalus*, were exposed to a concentration of 0.1 mg/l, 95% of the juveniles and 63% of the adults died within 48 hours. In 1.0 mg/l all juveniles were killed in 9 hours and adults in 15 hours (Lee *et al*., 1975). Merna and Eisele (1973) reported that methoxychlor is highly toxic to freshwater organisms and continuous exposure to 1.0 μg/l methoxychlor may cause serious sublethal effects. Methoxychlor in concentrations between 1.0 μg/l to 0.125 μg/l inhibited hatching of fathead minnow eggs and 2 μg/l prevented spawning. Concentrations of 50 μg/l inhibited phytoplankton production. When yellow perch were exposed to concentrations of methoxychlor from 0.625 to 6.00 μg/l for eight and a half months, growth was retarded as concentrations were increased. At 10.0 μg/l perch died. The 96 hour TL_{50} (tolerance limit for 50% survival) values for the amphipod, *Gammarus pseudolimaeus*, was 0.61 μg/l and 7.05 μg/l for the crayfish, *Orconectes virilis*.

There is a dearth of papers on the effect of methoxychlor on estuarine and marine decapods. Eisler (1969) determined the concentrations of seven organochloride and five organophosphorus insecticides that kill 50% of the sand shrimp, Crangon septemspinosa, the grass shrimp, Palaemonetes vulgaris, and the hermit crab, Pagurus longicarpus, in 96 hours. Methoxychlor was the third most toxic organochloride insecticide to Crangon septemspinosa, the fifth most toxic organochloride to Palaemonetes vulgaris and the third most toxic organochloride to Pagurus longicarpus. With few minor exceptions pesticide-induced mortality seemed to be related to temperature of the water. Mortality was least in the lowest test temperature of 10°C and greatest in the highest test temperature of 30°C.

Although methoxychlor might be considered a moderately hazardous organochloride insecticide, it may be highly toxic to marine organisms, especially near areas whre repeated applications of the insecticide are used. The Florida estuary from which we obtained ovigerous Rhithropanopeus harrisii in January and February, 1975, for experiments on malathion must have been such a place for four of the mother crabs had residues of methoxychlor ranging from 0.57 ppm to 5.50 ppm.

As far as known, there have been on investigations on the effect of methoxychlor on the complete larval development of crabs. The objectives of our present study were to determine the effects of methoxychlor on the complete larval development of Rhithropanopeus harrisii and Callinectes sapidus from the time of hatching until the 1st crab stage is reached. Specifically answers to the following questions will be sought: (1) what concentrations are sublethal and which are acutely toxic; (2) what are the sublethal effects of these concentrations; (3) what are the effects of methoxychlor on the development of the external and internal sex characters; (4) is there one or more larval stages particularly sensitive to different concentrations of methoxychlor; (5) what residues of methoxychlor are present in larvae of different ages in reference to concentration and length of exposure; and (6) what are the differences in the effects of methoxychlor on the complete development of R. harrisii and C. sapidus?

RESEARCH RESULTS

A. EFFECTS OF METHOXYCHLOR ON DEVELOPMENT OF Rhithropanopeus harrisii

Survival

Rhithropanopeus harrisii larvae hatched from four mother crabs, Rh I-IV, were reared in acetone control and in 1.0 ppb, 2.5 ppb, 4.0 ppb, 5.5 ppb, and 7.0 ppb methoxychlor. The percent survival of each of the four series of larvae which passed through zoeal and megalopa development is given in Table 7, and the average percent survival of larvae of four replicate series reared in six media is plotted in Figure 2. Survival of larvae from four crabs reared in acetone control through zoeal and megalopa development only varied from 98% to 100% and 96% to 100%, respectively (Table 7). Such high survival indicates that each of the four series of larvae used in the experiment were very healthy. In five concentrations of methoxychlor from 1.0 ppb to 7.0 ppb, there was differential survival (Figure 2). Survival through zoeal and through megalopa development to the 1st crab stage in 1.0 ppb methoxychlor was almost as high as in acetone control, whereas only 0.5% survived in 7.0 ppb, that is, only one larva out of 200 larvae in the four replicate series completed zoeal and megalopa development (Figure 2).

A two-way ANOVA with replication (Table 8) shows that there is no statistical difference in survival between the number of larvae which completed zoeal and megalopa development. The differences in survival in acetone, 1.0 ppb, 2.5 ppb and 4.0 ppb methoxychlor are highly significant ($P < 0.001$).

Duration of Development

Table 7 gives the mean duration of zoeal and megalopa development and the time from hatching to the 1st crab stage in each of four replicate series of larvae reared in six media. An average duration of zoeal and megalopa development of larvae from all replicate series is listed in Table 9. It shows that the duration of zoeal development is prolonged with each increase in concentration of methoxychlor from 1.0 ppb to 5.0 ppb. This is considered a sublethal effect of methoxychlor. The average duration of megalopa development in days, however, is similar in all experimental media (Table 9). The total times from hatch to 1st crab are prolonged with each increase in concentration, and therefore are related primarily to the effects of different concentrations of methoxychlor on zoeal development.

A two-way ANOVA with replication indicates that variation of duration of R. harrisii development is related to differences in zoeal and megalopa development, the media or treatment used and an interaction of the two, at the 0.001 level of probability (Table 10).

TABLE 7

EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Rhithropanopeus harrisii* I-IV

Culture Media Salinity 20‰ Temp. 25°C	Initial # of Larvae per Series	Mean Duration of			% Survival to	
		Zoeal Development in Days	Megalopa Development in Days	Hatching to 1st Crab in Days	Megalopa	1st Crab
Acetone Control	Rh I-50	10.86	4.63	15.48	98	96
	Rh II-50	11.04	6.04	17.08	100	100
	Rh III-50	9.98	4.80	14.78	98	98
	Rh IV-50	10.74	5.06	15.83	100	96
Methoyxchlor 1.0 ppb	Rh I-50	12.06	4.49	16.55	94	94
	Rh II-50	11.69	6.00	17.69	96	96
	Rh III-50	11.20	5.14	16.35	100	98
	Rh IV-50	11.31	5.57	16.89	96	92
Methoxychlor 2.5 ppb	Rh I-50	15.03	4.47	19.50	76	76
	Rh II-50	12.53	5.48	17.93	94	84
	Rh III-50	12.36	4.91	17.28	90	86
	Rh IV-50	12.51	5.17	18.67	78	72
Methoxychlor 4.0 ppb	Rh I-50	17.13	4.75	21.88	16	16
	Rh II-50	14.42	5.18	19.61	66	66
	Rh III-50	14.00	5.09	19.06	66	66
	Rh IV-50	14.63	5.28	19.91	64	64
Methoxychlor 5.5 ppb	Rh I-50	-	-	-	-	-
	Rh II-50	15.14	5.00	20.14	28	28
	Rh III-50	15.14	5.31	20.31	28	26
	Rh IV-50	15.64	5.09	20.73	22	22
Methoxychlor 7.0 ppb	Rh I-50	-	-	-	-	-
	Rh II-50	15.00	5.00	20.00	2	2
	Rh III-50	-	-	-	-	-
	Rh IV-50	-	-	-	-	-

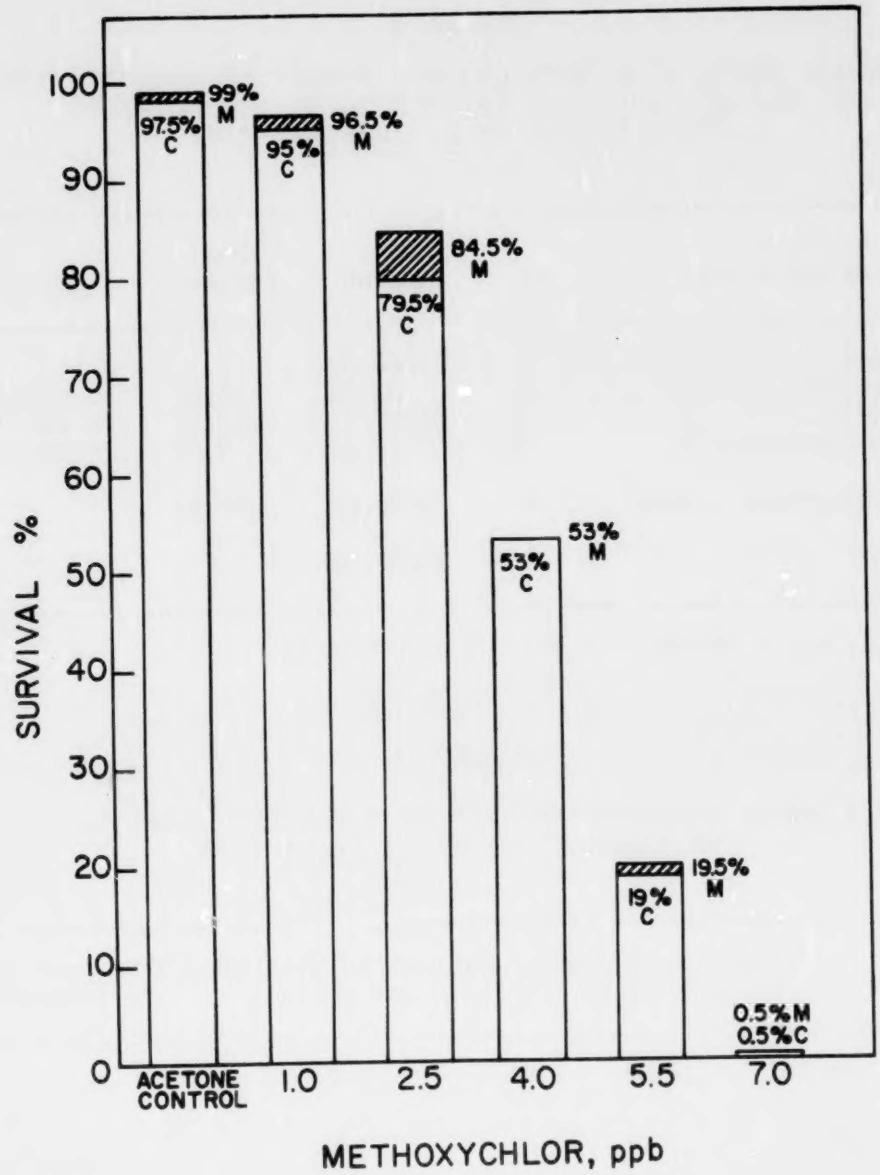

Figure 2. Average percent survival of four replicate series of Rhithropanopeus harrisii larvae, Rh I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of methoxychlor.

TABLE 8

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF R. harrisii I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	7	10410.00		
A (Stages)	1	32.00	32.00	0.1884 n.s.
B (Media)	3	10350.87	3450.29	20.316 ***
A x B (Interaction)	3	27.13	9.04	0.053 n.s.
Within subgroups (error)	24	4076.00	169.83	
Total	31	14486.00		

Significant F ratio: F.001 [3,24] = 7.55

TABLE 9

AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF R. harrisii I-IV

Medium	Duration of Zoeal Development	Duration of Megalopa Development	Time from Hatch to First Crab
Acetone Control	10.66	5.14	15.8
Methoxychlor			
1.0 ppb	11.56	5.30	16.87
2.5 ppb	13.04	5.01	18.30
4.0 ppb	14.56	5.15	20.17
5.5 ppb	15.28	5.13	20.37

Mortality

Rhithropanopeus harrisii passes through four zoeal stages and a megalopa, a fifth stage of development. In an effort

TABLE 10

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV

Source of Variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	7	490.28	70.04	
A (Stages)	1	445.73	445.73	730.70 ***
B (Media)	3	20.76	6.92	11.34 ***
A x B (Interaction)	3	23.79	7.93	13.00 ***
Within subgroups (error)	24	14.57	0.61	
Total	31	504.85		

Significant F ratios: $F_{.001}$ [1,24] = 14.0, $F_{.001}$ [3,24] = 7.55

to determine if larvae in one or more of the five developmental stages of R. harrisii were particularly sensitive to different concentrations of methoxychlor, a record of deaths by stage was made for larvae from four mother crabs, Rh I-IV, which had been reared in acetone control and five concentrations of methoxychlor (Table 11).

A two-way ANOVA with replication (Table 12) shows that the main effects of methoxychlor on the mortality of five development stages of R. harrisii are related to differences in stages, the media used and the interaction of the two ($P < 0.001$).

To determine which stage of development is siginificantly sensitive to a particular concentration of methoxychlor, a single classification ANOVA of mortality data was made for each medium in Table 11, using replicates from larvae of mother crabs, Rh I-IV. If the means of the series were significantly different, the least significant difference (LSD) was calculated. In acetone control, no single stage was significantly different ($t_{0.01}$) from any other. Larvae in zoeal stage I were significantly sensitive ($t_{0.01}$) to 1.0 ppb, 2.5 ppb and 4.0 ppb methoxychlor, as were larvae in zoeal stages I and II to 5.5 ppb and 7.0 ppb methoxychlor.

TABLE 11

PERCENT MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV

Stage		I	II	III	IV	Megalopa	Total
Acetone Control	Rh I	0	2	0	0	2	4
	Rh II	0	0	0	0	0	0
	Rh III	0	0	0	2	0	2
	Rh IV	0	0	0	0	4	4
Methoxychlor 1.0 ppb	Rh I	4	0	0	2	0	6
	Rh II	4	0	0	0	0	4
	Rh III	0	0	0	0	2	2
	Rh IV	0	0	0	2	2	4
Methoxychlor 2.5 ppb	Rh I	20	4	0	0	0	24
	Rh II	6	0	0	0	10	16
	Rh III	2	2	4	2	4	14
	Rh IV	20	0	0	2	6	28
Methoxychlor 4.0 ppb	Rh I	42	20	22	0	0	84
	Rh II	30	4	0	0	0	34
	Rh III	16	8	10	0	0	34
	Rh IV	20	10	4	0	0	36
Methoxychlor 5.5 ppb	Rh I	76	22	2	-	-	100
	Rh II	50	20	2	0	0	72
	Rh III	56	16	0	0	2	74
	Rh IV	40	38	0	0	0	78
Methoxychlor 7.0 ppb	Rh I	100	-	-	-	-	100
	Rh II	72	26	0	0	0	98
	Rh III	74	24	2	-	-	100
	Rh IV	68	28	2	2	-	100

TABLE 12

TWO-WAY ANOVA WITH REPLICATION; EFFECTS OF METHOXYCHLOR ON MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	29	36611.87	1262.48	
A (Stages)	4	14049.87	3512.47	96.21 ***
B (Media)	5	6650.67	1330.13	36.43 ***
A x B (Interaction)	20	15911.33	795.56	21.79 ***
Within Subgroups (error)	90	3286.00	36.51	
Total	119	39897.87		

Significant F ratios: F.001 [4,90] = 5.13,
F.001 [5,90] = 4.59,
F.001 [20,90] = 2.68

Residue Analysis

Larvae used in the experiments reported here were hatched from four ovigerous *Rhithropanopeus harrisii*, Rh I-IV. They were collected from the west side of Newfound Harbor, a small estuary which is between the Indian River to the west and the Banana River to the east in the vicinity of Cocoa Beach. Florida. To determine if the mother crabs came from a contaminated area in Florida, Hazleton Laboratories America, Inc., were engaged to analyze the crabs for chlorinated hydrocarbon pesticide residues (BHC, Lindane, Chlordane, Aldrin, Heptachlor Epoxide, Endrin, Dieldrin and HCB). None were found in detectable amounts (< 0.01 ppm) except Aroclor 1242 and Aroclor 1254. In Rh I-IV, Aroclor 1242 residues were 0.04 ppm, 0.29 ppm, 0.07 ppm and 0.07 ppm. Aroclor 12454 residues in Rh I-IV were 0.05 ppm, 0.018 ppm, 0.03 ppm and 0.04 ppm.

Analyses were also made to determine methoxychlor residues in *R. harrisii* larvae. No detectable methoxychlor (< 0.1 ppm) was found in newly hatched larvae, five and ten day larvae, megalopa and first crab stages reared in seawater control, or in acetone control in five and ten day larvae, megalopa and 1st crab stages. The results of the determination of methoxychlor residues in crab larvae reared in 1.0 ppb, 2.5 ppb, 4.0 ppb and 5.5 ppb are given in Table 13.

TABLE 13

METHOXYCHLOR RESIDUES IN *R. harrisii* LARVAE

Sample Identification and Treatment	Methoxychlor ppm
5 day larvae - 1.0 ppb Methoxychlor	< 0.1
10 day larvae - 1.0 ppb Methoxychlor	0.40
Megalopa - 1.0 ppb Methoxychlor	< 0.1
First Crab - 1.0 ppb Methoxychlor	< 0.1
5 day larvae - 2.5 ppb Methoxychlor	0.56
10 day larvae - 2.5 ppb Methoxychlor	0.51
Megalopa - 2.5 ppb Methoxychlor	1.12
First Crab - 2.5 ppb Methoxychlor	0.23
5 day larvae - 4.0 ppb Methoxychlor	lost
10 day larvae - 4.0 ppb Methoxychlor	0.59
Megalopa - 4.0 ppb Methoxychlor	0.55
First Crab - 4.0 ppb Methoxychlor	0.76
5 day larvae - 5.5 ppb Methoxychlor	1.28
First Crab - 5.5 ppb Methoxychlor	0.55

B. EFFECTS OF METHOXYCHLOR ON DEVELOPMENT OF *Callinectes sapidus*

Survival

Callinectes sapidus larvae hatched from four mother crabs (Cs I-IV) were reared in acetone control and in 0.7 ppb, 1.0 ppb, 1.3 ppb, 1.6 ppb and 1.9 ppb methoxychlor from the time of hatching through zoeal and megalopa development to the 1st crab stage. The percent survival of each of the four series of larvae which passed through zoeal and megalopa development is given in Table 14. The average percent survival of larvae of all replicate series reared in six media through zoeal and megalopa development is plotted in Figure 3. There is differential survival through zoeal and megalopa development which

TABLE 14

EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Callinectes sapidus* I-IV

Culture Media Salinity 30‰ Temp. 25°C	Initial # of Larvae per Series	Mean Duration of: Zoeal Development in Days	Megalopa Development in Days	Hatching to 1st Crab in Days	% Survival to: Megalopa	1st Crab
Acetone Control	Cs I-50	32.73	10.09	43.61	74	46
	Cs II-50	28.79	8.71	37.79	58	28
	Cs III-50	32.28	8.87	40.70	78	60
	Cs IV-50	35.20	9.17	44.38	80	80
Methoxychlor 0.7 ppb	Cs I-50	38.47	10.58	49.42	38	24
	Cs II-50	32.58	8.82	42.27	62	44
	Cs III-50	37.34	10.00	46.36	52	44
	Cs IV-50	37.97	10.00	48.35	66	62
Methoxychlor 1.0 ppb	Cs I-50	43.15	14.50	58.10	40	20
	Cs II-50	36.12	8.80	45.60	68	40
	Cs III-50	43.20	11.30	57.60	30	20
	Cs IV-50	40.67	12.77	53.38	36	26
Methoxychlor 1.3 ppb	Cs I-50	43.67	10.60	56.60	30	20
	Cs II-50	41.00	9.67	50.83	18	12
	Cs III-50	-	-	-	-	-
	Cs IV-50	37.50 (two)	8.00 (one)	52.00	4	2
Methoxychlor 1.6 ppb	Cs I-50	46.00	10.67	56.00	8	6
	Cs II-50	35.00 (two)	12.00 (one)	46.00	4	2
	Cs III-50	-	-	-	-	-
	Cs IV-50	-	-	-	-	-
Methoxychlor 1.9 ppb	Cs I-50	50.00 (one)	13.00 (one)	63.00	2	2
	Cs II-50	-	-	-	-	-
	Cs III-50	-	-	-	-	-
	Cs IV-50	-	-	-	-	-

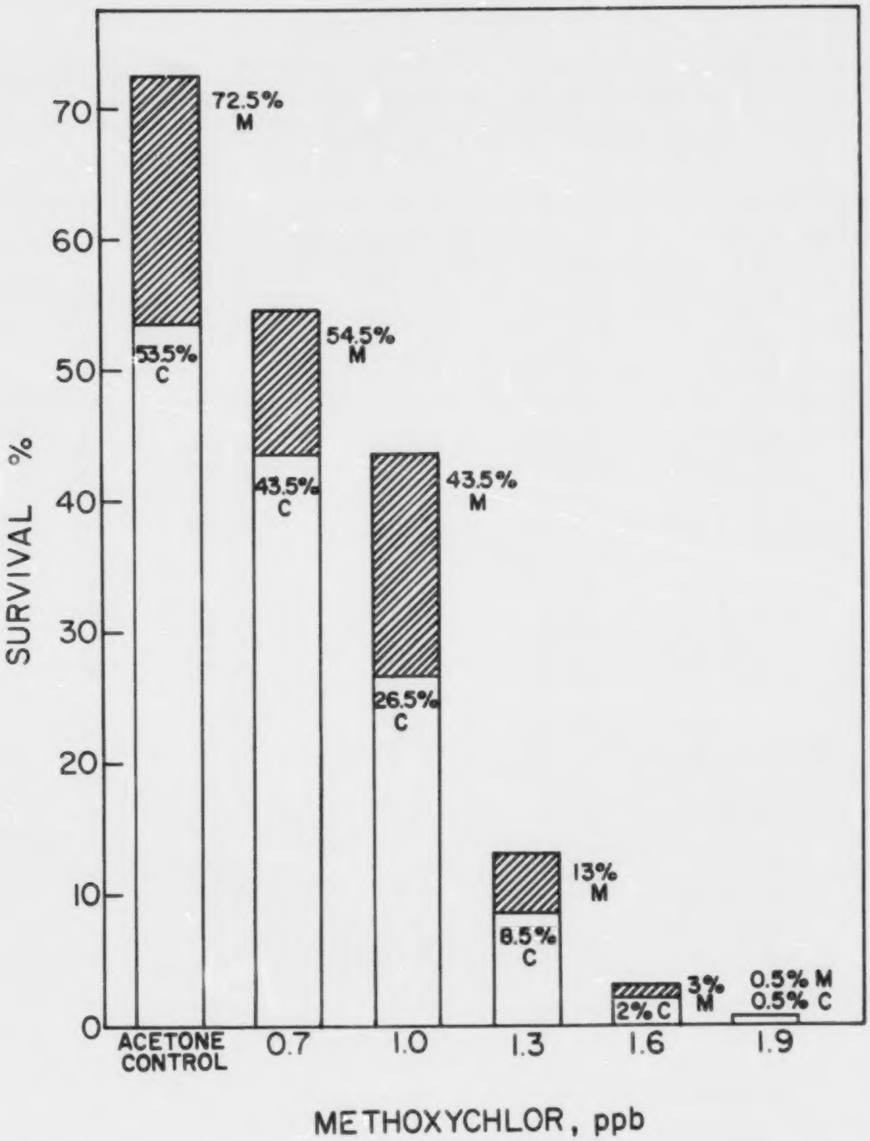

Figure 3. Average percent survival of four replicate series of Callinectes sapidus larvae, Cs I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of methoxychlor.

is related to increase in concentration of methoxychlor (Figure 3).

A two-way ANOVA with replication (Table 15) shows that the difference between survival to the megalopa stage and to the 1st crab stage is statistically significant at the 0.05 level of probability. The differences in survival related to media (i.e., acetone control, 0.7 ppb and 1.0 ppb methoxychlor) are significant at the 0.01 level. The interaction between stages and media is not significant statistically.

TABLE 15

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	5	4700.83	940.17	
A (Stages)	1	1441.50	1441.50	6.29 *
B (Media)	2	3192.33	1596.17	6.97 **
A x B (Interaction)	2	67.00	33.50	0.146 n.s.
Within subgroups (error)	18	4125.00	229.17	
Total	23	8825.83		

Significant F ratios: F.05 [1,18] = 4.41,
F.01 [2,18] = 6.01

Duration of Development

The mean duration of zoeal and megalopa development and the time from hatching to the 1st crab stage are given in Table 14 for larvae hatched from each mother crab, Cs I-IV, and reared in six media. The average duration of zoeal and megalopa development of all crabs, Cs I-IV, is shorter in acetone control than in any of the concentrations of methoxychlor (Table 16). In zoeal development and total time from hatching to 1st crab, the duration increases with concentration from 0.7 ppb to 1.3 ppb methoxychlor.

TABLE 16

AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV

Medium	Duration of Zoeal Development	Duration of Megalopa Development	Time from Hatch to First Crab
Acetone Control	32.50	9.22	42.32
Methoxychlor			
0.7 ppb	36.38	9.78	46.46
1.0 ppb	39.89	11.32	52.13
1.3 ppb	42.73	10.12	54.44
1.6 ppb	42.33	11.00	53.50
1.9 ppb	50.00 (one)	13.00 (one)	63.00 (one)

TABLE 17

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF METHOXYCHLOR ON DURATION OF ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	5	4292.28	858.46	
A (Stages)	1	4131.49	4131.49	709.88 ***
B (Media)	2	125.20	62.60	10.76
A x B (Interaction)	2	35.59	17.80	3.06 n.s.
Within subgroups (error)	18	104.75	5.82	
Total	23	4397.03		

Significant F ratios: $F_{.001}$ [1,18] = 15.4,
$F_{.001}$ [2,18] = 10.4

It was only in acetone control and in 0.7 ppb and 1.0 ppb that 20% or more of the larvae from each of the four mother crabs survived to both the megalopa and 1st crab stage (Table 14). A two-way ANOVA with replication of the data on duration of zoeal and megalopa development in these three media indicates that variation of duration of C. sapidus development is related to stage and to media at the 0.001 level of probability (Table 17). The interaction of stage and media is not significant statistically at P = 0.05.

Sexual morphogenesis

The differentiation of the external and internal sexual characters of R. harrisii and C. sapidus does not appear to be affected by different concentrations of methoxychlor. Sex ratio remains normal in all cases. Upon close examination of the pleopods of crab stages I through III, however, one to five plumose setae of the type characteristic of the megalopa stage were found at the tip of each crab pleopod. Furthermore, the pleopods of the male and female developed at a different rate in the higher concentrations of methoxychlor in both R. harrisii and C. sapidus than in acetone control.

Mortality

Callinectes sapidus may pass through seven, occasionally eight, zoeal stages before it molts into a megalopa, a ninth stage of development. In an effort to determine if there were one or more of the nine developmental stages of C. sapidus which were particularly sensitive to different concentrations of methoxychlor, a record of deaths by stage was made for larvae from each of the four mother crabs, Cs I-IV, which had been reared in acetone control and five concentrations of methoxychlor (Table 18).

A two-way ANOVA with replication (Table 19) shows that the main effects of methoxychlor on the mortality of nine developmental stages of C. sapidus are related significantly to differences in stages, the media used and interaction of the two.

To determine which stage of development is significantly sensitive to a particular concentration of methoxychlor, a single classification ANOVA of mortality data was made for each medium in Table 18, using replicates from larvae of mother crabs, Cs I-IV. If the means of the series were significantly different, the least significant difference was calculated. In

TABLE 18

PERCENT MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV

Stage		I	II	III	IV	V	VI	VII	VIII	Mega-lopa	Total
Acetone	Cs I	2	6	8	0	0	0	10	0	28	54
Control	Cs II	4	2	0	4	0	0	20	12	30	72
	Cs III	2	0	2	4	0	0	8	6	18	40
	Cs IV	8	4	0	2	2	0	2	2	0	20
Methoxy-	Cs I	0	4	16	4	0	2	36	0	14	76
chlor	Cs II	2	10	14	2	2	0	4	4	18	56
0.7 ppb	Cs III	6	20	16	0	2	0	4	0	8	56
	Cs IV	2	16	6	6	0	0	2	2	4	38
Methoxy-	Cs I	4	6	22	0	0	0	26	2	20	80
chlor	Cs II	4	8	8	2	0	4	2	4	28	60
1.0 ppb	Cs III	2	20	20	8	4	0	14	2	10	80
	Cs IV	8	16	26	8	2	0	4	0	10	74
Methoxy-	Cs I	12	0	40	6	0	0	12	0	10	80
chlor	Cs II	6	32	28	10	0	0	4	2	6	88
1.3 ppb	Cs III	6	78	16	-	-	-	-	-	-	100
	Cs IV	12	48	28	4	2	0	2	0	2	98
Methoxy-	Cs I	8	48	26	4	2	0	4	0	2	94
chlor	Cs II	2	42	48	4	0	0	2	0	0	98
1.6 ppb	Cs III	28	72	-	-	-	-	-	-	-	100
	Cs IV	26	70	4	-	-	-	-	-	-	100
Methoxy-	Cs I	26	40	30	0	0	0	2	0	0	98
chlor	Cs II	12	80	8	-	-	-	-	-	-	100
1.9 ppb	Cs III	36	64	-	-	-	-	-	-	-	100
	Cs IV	46	54	-	-	-	-	-	-	-	100

TABLE 19

TWO-WAY ANOVA WITH REPLICATION; EFFECTS OF METHOXYCHLOR ON MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* LARVAE

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	53	36493.87	688.56	
A (Stages)	8	12771.16	1596.40	22.29 ***
B (Media)	5	1119.20	223.84	3.13 **
A x B (Interaction)	40	22603.51	565.09	7.89 ***
Within subgroups (error)	162	11603.00	71.62	
Total	215	48096.87		

Significant F ratios: F.001 [8,162] = 3.46,
F.01 [5,162] = 3.13,
F.001 [40,162] = 2.03

acetone control a significantly greater number of deaths ($t_{0.01}$) occurred in the megalopa than in any other. No developmental stages were significantly different ($t_{0.01}$) from one another when the larvae were reared in 0.07 ppb and 1.0 ppb methoxychlor. Larvae in zoeal stages II and III were significantly sensitive statistically ($P < 0.01$) to 1.3 ppb methoxychlor, as were larvae in zoeal stage II to 1.6 ppb and zoeal stages I and II to 1.9 ppb methoxychlor.

Residue Analysis

Larvae used in the experiments reported here were hatched from four ovigerous *Callinectes sapidus*. The crabs were collected from the Beaufort Inlet, approximately two miles from Duke University Marine Laboratory, Beaufort, North Carolina. To determine if the mother crabs came from a contaminated area, Hazleton Laboratories were asked to make a determination of chlorinated hydrocarbon pesticide residues in mother crabs Cs I-IV and to determine methoxychlor residues in *C. sapidus* larvae.

No detectable (< 0.01 ppm) chlorinated hydrocarbon pesticide residues were found in crabs Cs I-IV of the following pesticides: BHC, Lindane, Chlordane, Aldrin, Heptachlor, Heptachlor Epoxide, DDE, DDD, DDT, Aroclor 1242, Aroclor 1254, Endrin, Dieldrin and HCB. Furthermore, no detectable (< 0.1 ppm) methoxychlor was found in newly hatched _C. sapidus_ larvae in seawater, megalopa and 1st crabs in seawater control, or in acetone control in 5 and 15 day larvae, megalopa and 1st crab stages. The results of determination of methoxychlor residues in crab larvae reared in 0.7 ppb, 1.0 ppb, 1.3 ppb, 1.6 ppb and 1.9 ppb methoxychlor are given in Table 20.

TABLE 20

METHOXYCHLOR RESIDUES IN _C. sapidus_ LARVAE

Sample Identification and Treatment	ppm Methoxychlor
5 day larvae - 0.7 ppb Methoxychlor	< 0.1
15 day larvae - 0.7 ppb Methoxychlor	< 0.1
Megalopa - 0.7 ppb Methoxychlor	0.51
First Crab - 0.7 ppb Methoxychlor	< 0.1
5 day larvae - 1.0 ppb Methoxychlor	0.52
10 day larvae - 1.0 ppb Methoxychlor	2.87
First Crab - 1.0 ppb Methoxychlor	0.15
5 day larvae - 1.3 ppb Methoxychlor	0.76
13 day larvae - 1.3 ppb Methoxychlor	2.62
First Crab - 1.3 ppb Methoxychlor	0.34
4 day larvae - 1.6 ppb Methoxychlor	1.25
First Crab - 1.6 ppb Methoxychlor	2.68
5 day larvae - 1.9 ppb Methoxychlor	0.81

DISCUSSION

Survival in methoxychlor

The range of concentrations in which differential survival of _Rhithropanopeus harrisii_ occurred from the time of hatching to the 1st crab stage was from 1.0 ppb to 7.0 ppb methoxychlor (Fig. 2), whereas the range for similar development of *Callinectes sapidus* was from 0.7 ppb to 1.9 ppb (Fig. 3). Since 95% of the *R. harrisii* larvae of the four series survived in 1.0 ppb, 79.5% in 2.5 ppb, 53% in 4.0 ppb and 19% in 5.5 ppb methoxychlor, these concentrations are considered sublethal. Only one larva survived to the megalopa and crab stage in one of the four series in 7.0 ppb methoxychlor. Therefore, this concentration is acutely toxic to *R. harrisii* larvae. The sublethal concentrations of methoxychlor for *C. sapidus* development were 0.7 ppb, in which there was 43.5% survival to the 1st crab stage, and 1.0 ppb, in which there was 26.5% survival. Survival to the 1st crab stage in 1.3 ppb, 1.6 ppb and 1.9 ppb was less than 10%, and hence, these concentrations are considered acutely toxic. Not only were *R. harrisii* larvae much more resistant to methoxychlor than *C. sapidus* larvae, but there was no significant difference in survival to the megalopa and to the 1st crab in *R. harrisii* development, but there was in *C. sapidus*. This means that megalopa of *R. harrisii* were not sensitive to methoxychlor but *C. sapidus* megalopa were (Figs. 2 and 3).

The differences in sensitivity to methoxychlor between the two species were marked, but the same species showed similar degrees of sensitivity when they were reared in a range of concentrations of mirex from 0.01 ppb to 10.0 ppb (Bookhout *et al.*, 1972; Bookhout and Costlow, 1975).

Young *Gammarus pseudolimnaeus* are more sensitive to methoxychlor than *R. harrisii* and *C. sapidus* larvae. At two, four and six weeks the TL_{50} in μg/1 was 0.30, 0.29 and 0.24, respectively. *Chironomus tentans*, however, when exposed to concentrations of 0.5 μg/1 to 2.0 μg/1 methoxychlor for 28 days, appear to be about as sensitive as *C. sapidus* larvae. Approximately 50% survived at 0.5 μg/1, 17% at 1.0 μg/1 and none at 2.0 μg/1. At concentrations of 0.125 μg/1 and 0.25 μg/1 more *C. tentans* survived and pupated or emerged than in the control media (Merna and Eisele, 1973).

Sublethal effects

The duration of zoeal development of *R. harrisii* was prolonged with each concentration of methoxychlor from 1.0 ppb to 5.0 ppb (Table 7), and there was a similar increase in duration of zoeal development of *C. sapidus* as concentrations of methoxychlor were increased from 0.7 ppb to 1.3 ppb (Table 14). The average duration of megalopa development was similar for both species in these concentrations, therefore, the total time for hatching to 1st crab was prolonged with each concentration primarily due to effects of methoxychlor on zoeal development (Tables 7 and 14). The reduction in molting rate is considered a sublethal effect of methoxychlor on both *R. harrisii* and *C. sapidus* zoeae. A similar increase in duration of zoeal development of *R. harrisii* with increase of concentration of mirex occurred (Bookhout *et al.*, 1972), but there was no significant prolongation of zoeal development of *C. sapidus* in sublethal concentrations of mirex, only in acutely toxic concentrations (Bookhout and Costlow, 1975).

During sexual morphogenesis from the megalopa to the 6th crab stage of *R. harrisii* and *C. sapidus*, methoxychlor has no apparent effect on the genital apparatus, but has sublethal effects on the pleopods. Since the sex ratio is normal in crabs reared in different concentrations of methoxychlor, this signifies that male or female sexual differentiation occurs normally. The dimorphism of abdominal appendages evolves according to the genetic sex. In males, only the gonopods, the 1st and 2nd pleopods, differentiate, whereas in females, pleopods 2-5 develop.

Natatory plumose setae, characteristic of megalopa, persist in crab stages I-III reared in different concentrations. This indicates an abnormal metamorphosis, since these setae completely disappear at the molt between megalopa and 1st crab stage in crabs reared in acetone control. Furthermore, male differentiation of pleopods is slower in crab stages I-VI reared in 4.0 ppb, 5.5 ppb and 7.0 ppb methoxychlor in the case of *R. harrisii* and 1.6 ppb and 1.9 ppb in the case of *C. sapidus*. The regression of the 3rd, 4th and 5th pair of pleopods occurs more slowly in the crabs reared in the above concentrations of methoxychlor than in the crabs reared in acetone. This delay in regression of 3rd, 4th and 5th pleopods and in loss of setae in treated crabs does not seem to be a handicap for further development of crabs for in the 4th, 5th and 6th crab stages the pleopods of the male resembled those in the control. In the female, the persistance of the megalopa setae on the last three pairs of pleopods is normal, they don't regress but develop.

Mortality

The main effects of methoxychlor on the mortality of larvae in four zoeal stages and a megalopa stage of *R. harrisii* and larvae in eight zoeal stages and a megalopa stage of *C. sapi-*

dus are related significantly (Tables 12 and 19) to differences in stages, media used and the interaction of the two.

A single classification ANOVA of mortality data for each medium in Table 11, and a calculation of the LSD of each, shows that there isn't any single stage of R. harrisii which is significantly different ($t_{0.01}$) from any other in acetone control. In 1.0 ppb, 2.5 ppb and 4.0 ppb methoxychlor, however, stage I zoeae were significantly sensitive ($t_{0.01}$), and in 5.5 ppb and 7.0 ppb methoxychlor, larvae in zoeal stages I and II were sensitive statistically. Mirex, on the other hand, in concentrations of 0.01 ppb, 0.1 ppb, 1.0 ppb and 10.0 ppb seldom had any effect on larvae in zoeal stage I of the same species (Bookhout et al., 1972).

Larvae in zoeal stage I of C. sapidus were not significantly sensitive to any of the concentrations of methoxychlor used except the highest, 1.9 ppm. In acetone control, a significantly greater ($t_{0.01}$) number of deaths occurred in the megalopa than in any of the eight zoeal stages. No developmental stages were significantly different ($t_{0.01}$) from any other when reared in the sublethal concentrations of 0.07 ppb and 1.0 ppb methoxychlor. In the acutely toxic concentration of 1.3 ppb, larvae in stages II and III were significantly sensitive ($t_{0.01}$), as were larvae in zoeal stage II to 1.6 ppb and larvae in zoeal stages I and II to 1.9 ppb.

When two series of C. sapidus larvae were reared in acetone control and four concentrations of mirex, there were no stages which were significantly sensitive in acetone control or to 0.01 ppb mirex. By contrast, larvae in zoeal stages III, VII and megalopa in 0.1 ppb mirex and larvae in zoeal stages II and III in 1.0 ppb mirex showed significantly greater mortality than did larvae in other stages. In 10.0 ppb mirex, all larvae died in zoeal stages I and II.

Residues

Biological magnification of methoxychlor at 5 days and 10-15 days is much greater in C. sapidus than R. harrisii. There is no evidence from the limited data available that residues at any one concentration increase with age of larvae (Tables 13 and 20). In R. harrisii, there is an increase of residue with concentration at five and ten days, but this trend is not shown in megalopa and in 1st crab. In C. sapidus, there is an increase in residue and biological magnification with concentration in 5 day larvae and in 1st crabs which had been reared in 1.0 ppb, 1.3 ppb and 1.6 ppb methoxychlor.

Lee *et al*. (1975) reported that small amounts of methoxychlor, 0.1 μg/g in juveniles and 0.2 μg/g in adults, accumulated in striped mullets which had been exposed to 0.01 mg/l methoxychlor for 96 hours. Juveniles or adults exposed to 0.1, 1.0, and 10.0 mg/l for 96 hours did not have significantly different residues, although adults carried a heavier load of methoxychlor than juveniles.

Kennedy *et al*. (1970) made residue analyses for methoxychlor of whole bluegills from an untreated pond and two treated ponds on day 1, 3, 7, 14, 28, 56 and 84. No residues were detected in fish taken from untreated ponds. Fish from ponds treated with 0.01 ppm methoxychlor had residues of 2.11 ppm and 2.78 ppm on day one, peaked at 7 days with residues of 3.35 ppm and 4.00 ppm, and had no detectable residues at 56 or 84 days. Fish from ponds treated with 0.04 ppm methoxychlor had residues of 9.80 ppm and 13.90 ppm at day one and peaked at 3 days with 20.60 ppm and 21.10 ppm and no residues were detected at 56 and 84 days.

After snails, mosquitoes and fish had been exposed to 1.6 ppb methoxychlor for 28 days, residues of methoxychlor were 15.7 ppm in a snail, 0.48 ppm in mosquito, and 0.33 ppm in the fish, *Gambusia* (Metcalf *et al*., 1971).

If the biologial magnification of methoxychlor were calculated for examples cited, the snail exhibited the highest rate. It apparently could not metabolize methoxychlor rapidly, and as a result stored it at a substantial level (Metcalf *et al*., 1971). The biological magnification of methoxychlor in developmental stages of *C. sapidus* was generally above that reported in the literature for fish with peaks reaching 2870 and 2015.6 times the medium in which they were reared. The rate of biological magnification of methoxychlor of fish at peak periods was somewhat above (Kennedy *et al*., 1970) that found for *R. harrisii* larvae.

SECTION VII

MALATHION

INTRODUCTION

Malathion, like many other organophosphate antichloinesterase insecticides, is used widely to control crop pests, flies and mosquitoes. The estimated production of malathion in the United States was 24 million pounds in 1972 (EPA-540/1-75-005). The use of chlorinated hydrocarbon insecticides are being questioned because of their persistence and the residues which are concentrated through biological accumulation. The current trend, therefore, will probably be to replace chlorinated hydrocarbons with less persistent organophosphates, including malathion, and carbamate pesticides (Coppage and Duke, 1971).

The toxicity of malathion to aquatic organisms apparently depends upon the exposure time, temperature, pH of the water, and the species. Malathion decomposes at high temperatures. It is reported to be quite stable under neutral or acid pH conditions. Susceptibility to hydrolysis increases, however, with increasing alkalinity (Walker and Stojanovic, 1973). At pH 5 to 7, no hydrolysis could be detected after 12 days; at pH 9, 50% hydrolysis occurs in 12 hours, and at pH 12, hydrolysis takes place immediately (EPA-540/1-75-005). Malathion is rapidly dissipated in non-sterile soils due to microflora in the soil and chemical mechanisms (Walker and Stojanovic, 1973). In the absence of residues of malathion in animals from treated areas, measurements of the enzyme acetylchlinesterase (AChE) are probably the best general indicator of organophosphate pesticide pollution (Coppage and Matthews, 1974). Inhibition of AChE is believed to be the cause of death of higher vertebrates by blocking neurotransmission in the respiratory center of the brain or neuromuscular junctions of the respiratory apparatus. Inhibition of AChE is also thought to be the mode of action of these pesticides on arthropods (O'Brien, 1967).

Even though malathion is biodegradable, it may be highly toxic to target and non-target organisms alike. As a non-persistent insecticide, repeated applications of malathion may be necessary for control of pests, and cumulative reduction of AChE may occur (Coppage and Matthews, 1974). Furthermore, there is documented evidence from the literature that arthropod pests develop resistance to malathion (Mengle and Lewallen, 1963; Busvine, 1959; Brown and Abedi, 1960; La Brecque and Wilson, 1960; Mount, Seawright and Pierce, 1974). Hence, increasing concentrations of malathion have to be used to control pests, and the danger to non-target organisms becomes greater.

Few laboratory and field studies have been conducted on the effects of malathion on estuarine animals. Darsie and Corriden (1959) reported that malathion formulated in fuel oil and sprayed by airplane [560 g/ha (8 wt.oz/acre)] on tidal marshes in Delaware was toxic within four hours to *Fundulus ocellaris* held in tubs. Tagatz *et al*. (1974) studied the effects of thermal fog 420 g/ha (6 wt.oz/acre) and ULV aerosol spray 57 g/ha (0.64 fl.oz/acre) applications of malathion 95 on salt marsh environments in northwestern Florida. After three treatments deaths due to malathion were not observed to confined blue crabs, *Callinectes sapidus*; grass shrimps, *Palaemonetes vulgaris* and *P. pugio*; pink shrimp, *Penaeus duorarum*; or sheepshead minnows, *Cyprinodon variegatus*. Conte and Parker (1971), however, reported that an aerial application of (256 g/ha) 3 fl. oz/acre to marsh embayments in Texas killed 14 to 80% of commercial shrimp, *Penaeus aztecus* and *P. setiferus*. They found residues of malathion of 0.8 to 3.2 ppm in the water and in the tissues of living shrimp residues from 0.28 to 2.67 ppm up to 48 hours after spraying.

To date, however, there have been no publications on the effects of malathion on the larval development of crabs. If aerial spraying of malathion can kill 14 to 80% of commercial shrimp, similar treatment could have sublethal and/or lethal effects on the larval development of crabs.

The objective of our study was to determine the effects of malathion on the complete larval development of *Rhithropanopeus harrisii* and *Callinectes sapidus* from the time of hatching until the 1st crab stage is reached. Specifically answers to the following questions will be sought: (1) what concentrations of malathion are sublethal and which are acutely toxic; (2) what are the sublethal effects of these concentrations; (3) what are the effects of malathion on the development of the external and internal sex characters; (4) is there one or more larval stages particularly sensitive to different concentrations of malathion; and, (5) what are the differences in the effects of malathion on the complete development of *R. harrisii* and *C. sapidus*?

RESEARCH RESULTS

A. EFFECTS OF MALATHION ON DEVELOPMENT OF *Rhithropanopeus harrisii*

Survival

Rhithropanopeus harrisii larvae hatched from mother crabs, ~~Rh I-IV, were reared in~~ acetone control and in 0.011 ppm, 0.014 ppm, 0.017 ppm, 0.02 ppm and 0.05 ppm malathion. The percent survival of four series of larvae, which passed through zoeal and megalopa development, is given in Table 21, and the average percent survival of larvae of four replicate series in five media is plotted in Figure 4. Since 97.5% of the larvae survived zoeal development and reached the megalopa stage in acetone control and 94.5% of the megalopa molted to the 1st crab stage, it can be concluded that the larvae used in the experiment were very healthy. There was a reduction in survival of larvae with each increase in concentration of malathion from 0.011 ppm to 0.02 ppm (Fig. 4). Larvae reared in 0.05 ppm did not survive beyond the second zoeal stage.

A two-way ANOVA with replication, Table 22, shows that the differences between the number of larvae which completed zoeal and megalopa development and differences in survival in acetone control and in 0.011 ppm, 0.014 ppm, 0.017 ppm and 0.02 ppm are both highly significant (0.001 probability).

Duration of Development

Table 21 gives the mean duration of zoeal and megalopa development and the mean time from hatching to the 1st crab stage in each of the four replicate series of larvae reared in five media. The average duration of zoeal and megalopa development of larvae from four replicate mother crabs is tabulated in Table 23. The duration of zoeal development is shortest in acetone control, and is lengthened with each increase in concentration of malathion from 0.011 ppm to 0.02 ppm. The duration of the megalopa stage in the five media does not follow this trend, but the total time from hatching to the 1st crab stage does (Table 23).

TABLE 21

EFFECT OF MALATHION ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Rhithropanopeus harrisii* I-IV

Culture Media Salinity 20‰ Temp. 25°C	Initial # of Larvae per Series	Mean Duration of			% Survival to	
		Zoeal Development in Days	Megalopa Development in Days	Hatching to 1st Crab in Days	Megalopa	1st Crab
Acetone Control	Rh I-50	10.92	4.96	15.88	100	100
	Rh II-50	11.38	5.28	16.66	100	100
	Rh III-50	10.73	5.47	16.20	98	98
	Rh IV-50	11.02	6.00	16.63	92	80
Malathion 0.011 ppm	Rh I-50	11.46	4.79	16.30	88	78
	Rh II-50	11.26	5.20	16.43	84	60
	Rh III-50	11.37	5.00	16.18	82	66
	Rh IV-50	11.24	5.21	16.35	84	68
Malathion 0.014 ppm	Rh I-50	11.47	5.00	15.96	68	48
	Rh II-50	10.95	5.13	16.50	44	16
	Rh III-50	11.52	4.88	16.11	44	36
	Rh IV-50	11.63	5.36	16.86	38	28
Malathion 0.017 ppm	Rh I-50	12.21	5.13	18.00	38	16
	Rh II-50	10.95	5.13	16.50	30	10
	Rh III-50	12.57	6.00	17.00	14	2
	Rh IV-50	12.56	4.60	17.40	18	10
Malathion 0.02 ppm	Rh I-50	12.67	5.50	17.00	6	2
	Rh II-50	12.00	-	-	6	0
	Rh III-50	12.00	-	-	2	0
	Rh IV-50	-	-	-	0	0

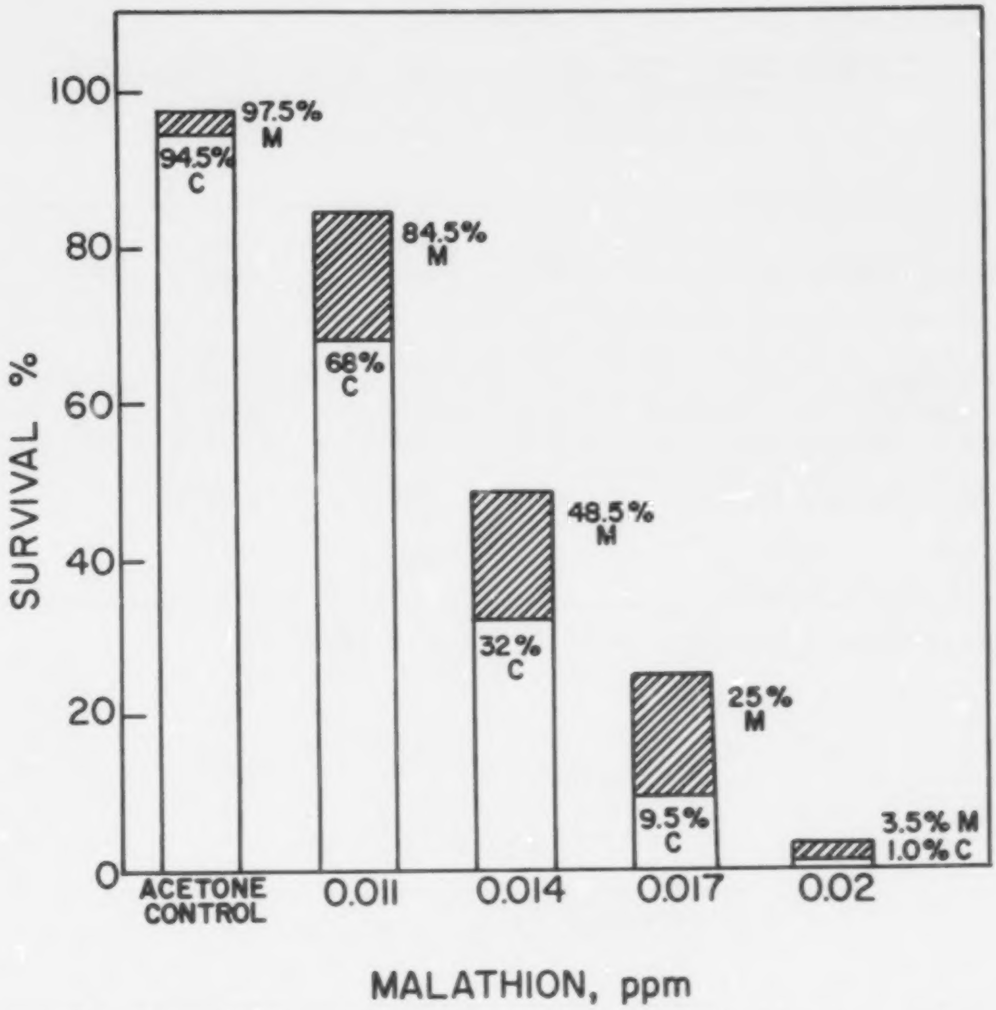

Figure 4. Average percent survival of four replicate series of Rhithropanopeus harrisii, Rh I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of malathion.

TABLE 22

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	9	51103.6	5678.2	
A (Stages)	1	1166.4	1166.4	16.74 ***
B (Media)	4	49378.6	12344.7	177.19 ***
A x B (Interaction)	4	558.6	139.7	2.01 n.s.
Within subgroups (error)	30	2090.0	69.67	
Total	39	53193.6		

Significant F ratios: F.001 [1,30] = 13.3,
F.001 [4,30] = 6.12

TABLE 23

AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV

Medium	Duration of Zoeal Development	Duration of Megalopa Development	Time from Hatch to First Crab
Acetone Control	11.01	5.43	16.19
Malathion			
0.011 ppm	11.33	5.05	16.32
0.014 ppm	11.39	5.09	16.36
0.017 ppm	12.07	5.22	17.23
0.02 ppm	12.22	5.50 (one)	17.00 (one)

A two-way ANOVA with replication indicates that variation of duration of R. harrisii larvae reared in acetone control and four concentrations of malathion is related to stages and to media at the 0.001 and 0.05 level of probability, respectively (Table 24).

TABLE 24

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	9	494.94	55.10	
A (Stages)	1	430.79	430.79	92.10 ***
B (Media)	4	60.75	15.19	3.25 *
A x B (Interaction)	4	4.40	1.10	0.24 n.s.
Within subgroups (error)	30	140.15	4.67	
Total	39	636.09		

Significant F ratios: F.001 [1,30] = 13.3,
F.05 [4,30] = 2.69

Sexual Morphogenesis

Malathion in the higher concentrations had no effect on the development of the genital apparatus of *R. harrisii* and *C. sapidus* in crab stages I-VI. It had the sublethal effect in the male of retarding the loss of pleopods 3-5 and the loss of megalopa setae after metamorphosis, as was described previously for methoxychlor.

Effect of Malathion on Mortality

Rhithropanopeus harrisii passes through four zoeal stages and a megalopa. In an effort to determine if larvae in one or more stages of development were particularly sensitive to different concentrations of malathion, a record of deaths by stage was made for larvae reared from four mother crabs, Rh

I-IV (Table 25).

TABLE 25

PERCENT MORTALITY IN FIVE DEVELOPMENTAL STAGES OF *R. harrisii* I-IV

Stage		I	II	III	IV	Megalopa	Total
Acetone Control	Rh I	0	0	0	0	0	0
	Rh II	0	0	0	0	0	0
	Rh III	0	0	0	2	0	2
	Rh IV	2	0	0	6	12	20
Malathion 0.011 ppm	Rh I	2	8	0	2	10	22
	Rh II	6	6	4	0	24	40
	Rh III	0	12	2	4	16	34
	Rh IV	2	14	0	0	16	32
Malathion 0.014 ppm	Rh I	2	22	4	4	20	52
	Rh II	6	18	4	28	28	84
	Rh III	0	14	12	30	8	64
	Rh IV	0	44	8	10	10	72
Malathion 0.017 ppm	Rh I	0	58	0	4	22	84
	Rh II	8	32	12	18	20	90
	Rh III	0	50	14	22	12	98
	Rh IV	2	74	0	6	8	90
Malathion 0.02 ppm	Rh I	2	82	8	2	2	96
	Rh II	0	60	26	8	6	100
	Rh III	14	70	12	2	2	100
	Rh IV	0	92	8	0	0	100
Malathion 0.05 ppm	Rh I	80	20	-	-	-	100
	Rh II	30	70	-	-	-	100
	Rh III	98	2	-	-	-	100
	Rh IV	80	20	-	-	-	100

A two-way ANOVA with replication of the data in Table 25 shows that the main effects of malathion on mortality of five larval stages of *R. harrisii* are related to stages, media and interaction of the two at the 0.001 level of probability (Table 26).

TABLE 26

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON THE MORTALITY IN FIVE LARVAL STAGES OF *R. harrisii*

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	29	46838.67	1615.13	
A (Stages)	4	11819.00	2954.75	28.56 ***
B (Media)	5	6132.27	1226.45	11.86 ***
A x B (Interaction)	20	28887.40	1444.37	13.96 ***
Within subgroups (error)	90	9310.00	103.44	
Total	119	56148.67		

Significant F ratios: $F_{.001}$ [4,90] = 5.13,
$F_{.001}$ [5,90] = 4.59,
$F_{.001}$ [20,90] = 2.68

To determine which stage of development is significantly sensitive to a particular concentration of malathion, a single classification ANOVA of the mortality data was made for each medium in Table 25, using replicates from larvae of mother crabs, Rh I-IV. If the means of the series were significantly different, the least significant difference was calculated. In acetone control, no single stage was significantly different in mortality from any other. Larvae in zoeal stage II and megalopa were significantly sensitive statistically ($t_{0.01}$) to 0.011 ppm malathion, as were larvae in zoeal stage II to 0.014 ppm, 0.017 ppm and 0.02 ppm, and larvae in zoeal stage I to 0.05 ppm malathion.

Residue Analysis

Since Hazleton Laboratories in 1973 found no detectable malathion in samples of blue crab larvae reared in concentrations ranging from 0.1 ppb to 100.0 ppb, it was not deemed advisable by Mr. Jack Lowe, the project director, to have *Rhithropanopeus* larvae analyzed for residues of malathion. The four mother crabs, Rh I-IV, which furnished the larvae for the investigation in 1975 were analyzed, however, for pesticides by Hazleton Laboratories in May, 1975. No Lindane, Chlordane, Aldrin, Heptachlor, Heptachlor Epoxide, DDE or DDD were detected. The results determined for these pesticides in the 6/94 ethyl ether/petroleum ether fraction could not be detected lower than 0.05 ppm due to the interference of Aroclor 1242 peaks. No Aroclor (<.5 ppm), Mirex (<.2 ppm), Dieldrin (<.01 ppm) or Endrin (<.01 ppm) was found either. The pesticides which were detected are given in Table 27.

TABLE 27

RESIDUES IN FOUR MOTHER CRABS, Rh I-IV

Pesticide in ppm	Rh I	Rh II	Rh III	Rh IV
DDT	0.19	0.08	0.37	0.32
Aroclor 1242	1.75	0.57	2.32	0.70
Methoxychlor	0.57	1.56	5.50	0.80

RESEARCH RESULTS

B. EFFECTS OF MALATHION ON DEVELOPMENT OF Callinectes sapidus

Survival

Callinectes sapidus larvae hatched from mother crabs, Cs I-IV, were reared in acetone control, and in 0.02 ppm, 0.05 ppm, 0.08 ppm and 0.11 ppm malathion. The percent survival of four series of larvae which completed zoeal and megalopa development is given in Table 28. The average percent survival of larvae from four replicate series reared in five media is plotted in Figure 5. Survival to megalopa and 1st crab stage in all concentrations of malathion was less than in acetone control. There was differential survival in concentrations of malathion from 0.02 ppm to 0.08 ppm (Fig. 5). Survival in 0.11 ppm malathion was somewhat better than in 0.08 ppm but lower than in 0.05 ppm. This apparent discrepancy will be considered later in the discussion.

TABLE 28

EFFECT OF MALATHION ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *Callinectes sapidus* I-IV

Culture Media Salinity 30‰ Temp. 25 C°	Initial # of Larvae per Series	Mean Duration of			% Survival to	
		Zoeal Development in Days	Megalopa Development in Days	Hatching to 1st Crab in Days	Megalopa	1st Crab
Acetone Control	Cs I-50	40.81	7.82	48.41	42	34
	Cs II-50	35.45	7.64	43.09	66	66
	Cs III-50	37.17	7.42	44.36	72	66
	Cs IV-50	35.68	8.12	44.00	56	52
Malathion 0.02 ppm	Cs I-50	41.84	10.38	51.88	38	32
	Cs II-50	37.68	8.33	45.88	50	48
	Cs III-50	41.43	10.33	52.67	14	12
	Cs IV-50	38.70	7.90	46.60	40	40
Malathion 0.05 ppm	Cs I-50	44.38	11.57	56.29	16	14
	Cs II-50	39.30	9.50	48.80	20	20
	Cs III-50	40.25	9.75	50.00	8	8
	Cs IV-50	40.67	8.47	49.13	30	30
Malathion 0.08 ppm	Cs I-50	48.00	10.50	55.50	6	4
	Cs II-50	46.33	8.66	55.00	6	6
	Cs III-50	43.00	8.00	49.00	6	4
	Cs IV-50	43.00	7.00	50.00	8	8
Malathion 0.11 ppm	Cs I-50	41.00	7.80	48.80	10	10
	Cs II-50	45.25	7.88	53.13	16	16
	Cs III-50	47.25	9.00	56.33	8	6
	Cs IV-50	44.00	7.20	51.20	10	10

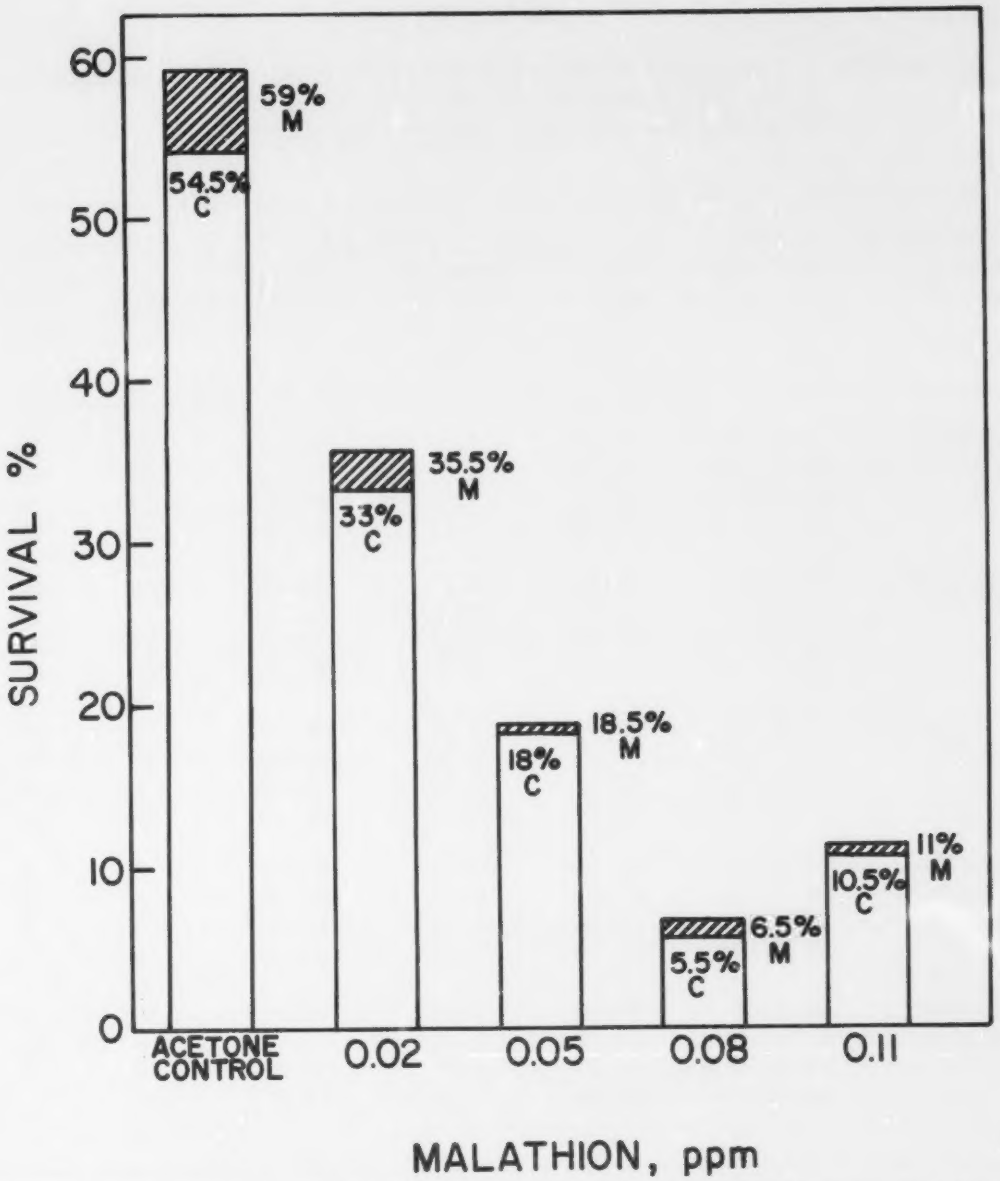

Figure 5. Average percent survival of four replicate series of Callinectes sapidus, Cs I-IV, reared from hatching to megalopa (M) and to 1st crab (C) in different concentrations of malathion.

A two-way ANOVA with replication (Table 29) shows that the variation in media accounted for the differences in survival ($P < 0.001$). The stage and interaction between stages and media were not significant.

TABLE 29

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON PERCENT SURVIVAL THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF C. sapidus I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	9	13680.4	1520.04	
A (Stages)	1	32.4	32.40	0.30 n.s.
B (Media)	4	13624.4	3406.10	31.56 ***
A x B (Interaction)	4	23.6	5.90	0.05 n.s.
Within subgroups (error)	30	3238.0	107.93	
Total	39	16914.4		

Significant F ratio: $F_{.001}[4,30] = 6.12$

Duration of Development

Table 28 shows the variation in mean duration of zoeal and megalopa development and the time from hatching to the 1st crab stage in each of the four replicate series of larvae reared in five media. The average duration of zoeal and megalopa development of larvae from four replicate mother crabs is tabulated in Table 30. The duration of zoeal development is shortest in acetone control, and is lengthened with each increase in concentration of malathion from 0.02 ppm to 0.08 ppm. The duration of the megalopa stage does not follow this trend, but the total time from hatching to the 1st crab stage does (Table 30).

A two-way ANOVA with replication (Table 31) reveals that variation of duration of *C. sapidus* larvae reared in acetone control and four concentrations of malathion is related to stages, media and interaction of the two at the 0.001 level of probability.

TABLE 30

AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV

Medium	Duration of Zoeal Development	Duration of Megalopa Development	Time from Hatch to First Crab
Acetone Control	36.98	7.72	44.52
Malathion			
0.02 ppm	39.45	8.85	48.16
0.05 ppm	41.05	9.50	50.53
0.08 ppm	44.92	8.27	52.18
0.11 ppm	44.36	7.86	52.10

TABLE 31

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF *C. sapidus* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	9	10999.68	1222.19	
A (Stages)	1	10821.47	10821.47	3136.66 ***
B (Media)	4	89.54	22.39	6.49 ***
A x B (Interaction)	4	88.67	22.17	6.43 ***
Within subgroups (error)	30	103.61	3.45	
Total	39	11103.29		

Significant F ratios: $F_{.001}[1,30] = 13.3$,
$F_{.001}[4,30] = 6.12$

Effect of Malathion on Mortality

Callinectes sapidus usually passes through seven zoeal stages and a megalopa stage before reaching a first crab stage. A frequent variation from the norm in the life history of the blue crab is the addition of an 8th zoeal stage before a molt to a megalopa stage. To determine if larvae in one or more of the nine stages of development of *C. sapidus* were particularly sensitive to different concentrations of malathion, a record of deaths by stage was made for larvae reared from four mother crabs, Cs I-IV (Table 32).

TABLE 32

PERCENT MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV

Stage		I	II	III	IV	V	VI	VII	VIII	Megalopa	Total
Acetone Control	Cs I	2	50	2	0	0	0	4	0	8	66
	Cs II	0	4	20	8	0	2	0	0	0	34
	Cs III	8	14	6	0	0	0	0	0	6	34
	Cs IV	2	24	14	0	0	0	4	0	4	48
Malathion 0.02 ppm	Cs I	4	30	16	4	6	0	2	0	6	68
	Cs II	8	28	12	2	0	0	0	0	2	52
	Cs III	4	44	22	4	0	4	6	2	2	88
	Cs IV	4	22	30	2	0	0	0	2	0	60
Malathion 0.05 ppm	Cs I	0	52	28	2	0	0	0	2	2	86
	Cs II	16	42	20	2	0	0	0	0	0	80
	Cs III	20	46	14	4	0	0	4	4	0	92
	Cs IV	4	22	38	6	0	0	0	0	0	70
Malathion 0.08 ppm	Cs I	18	72	4	0	0	0	0	0	2	96
	Cs II	22	40	30	0	2	0	0	0	0	94
	Cs III	16	42	32	4	0	0	0	0	2	96
	Cs IV	14	42	34	0	0	2	0	0	0	92
Malathion 0.11 ppm	Cs I	44	44	0	0	0	0	0	2	0	90
	Cs II	46	28	10	0	0	0	0	0	0	84
	Cs III	48	40	2	0	0	0	2	0	2	94
	Cs IV	68	10	8	4	0	0	0	0	0	90

A two-way ANOVA with replication of the data in Table 32 shows that the main effects of malathion on mortality are associated significantly with stages, media and interaction of the two (Table 33).

TABLE 33

TWO-WAY ANOVA WITH REPLICATION; EFFECT OF MALATHION ON MORTALITY IN NINE DEVELOPMENTAL STAGES OF *C. sapidus* I-IV

Source of variation	DF	Sum of Squares	Mean Square	Fs
Subgroups	44	32910.58		
A (Stages)	8	23580.58	2947.50	69.68 ***
B (Media)	4	698.36	174.59	4.12 **
A x B (Interaction)	32	8631.64	269.74	6.38 ***
Within subgroups (error)	135	5711.00	42.30	
Total	179	38621.58		

Significant F ratios: F.001 [8,135] = 3.51,
F.005 [4,135] = 3.89,
F.001 [32,135] = 2.19

To ascertain which stage of development is significantly sensitive to a particular concentration of malathion a single classification ANOVA of the mortality data was made for each medium in Table 32, using replicates from larvae of mother crabs, Cs I-IV. If the means of the series were significantly different, the least significant difference was calculated. Zoeal stage II was significantly different from the other developmental stages in acetone control, hence a two-way ANOVA of stages II and III was performed to compare the malathion concentrations with an acetone control. From all calculations no stages were significantly sensitive to 0.02 malathion. Larvae in zoeal stages II and III were significantly sensitive statistically ($P < 0.05$) to 0.05 ppm malathion, as were larvae in zoeal stages I, II and III to 0.08 ppm and larvae in stage I to 0.11 ppm malathion.

DISCUSSION

Survival in Malathion

The range of concentrations in which differential survival of Rhithropanopeus harrisii occurred from the time of hatching to the 1st crab stage was from 0.011 ppm to 0.02 ppm malathion (Fig. 4), whereas the range for similar development of Callinectes sapidus was from 0.02 ppm to 0.11 ppm malathion (Fig. 5). Of the four concentrations in which there was survival to the 1st crab stage, in R. harrisii two were sublethal, 0.011 ppm and 0.014 ppm, and two were acutely toxic, 0.017 ppm and 0.02 ppm (Fig. 4). In 0.02 ppm malathion, there was a small number of larvae which reached the megalopa stage in three of the four series, but only two larvae reached the 1st crab stage in one of the four series (Table 21). Larvae reared in 0.05 ppm malathion did not survive beyond the 2nd zoeal stage. In C. sapidus there was survival to the 1st crab stage in each concentration of each of the replicate series of larvae. Survival of larvae to the 1st crab stage varied in the replicate series reared in acetone control. Nevertheless, if the number of larvae which survived to megalopa and to 1st crab is noted for the same replicate, it can be seen that there is a reduction in survival in concentrations from 0.02 ppm to 0.08 ppm. Survival in 0.11 ppm was slightly higher than in 0.08 ppm. Concentrations of 0.02 ppm and 0.05 ppm were sublethal, and 0.08 ppm and 0.11 ppm were acutely toxic.

A two-way ANOVA shows that the differences in survival of R. harrisii are related to stages ($P < 0.001$) and media ($P < 0.05$) used (Table 22), but the differences in survival to the 1st crab stage of C. sapidus are mainly associated with the variation in media ($P < 0.001$). Stage and interaction between stage and media were not significant (Table 29).

When R. harrisii was exposed to different concentrations of methoxychlor throughout larval development, there was no statistical difference between the number of larvae which completed zoeal and megalopa development, but the variations in survival in different media were highly significant (Table 8), whereas the variations in survival in C. sapidus development were related to differences between survival to the megalopa stage and to the 1st crab stage, as well as to media (Table 15). This is just the opposite to the reaction of the larvae of the two species to malathion.

The developmental stages of C. sapidus are much more resistant to malathion than the larvae of R. harrisii. A concentration of 0.02 ppm malathion was sublethal to C. sapidus but acutely toxic to *R. harrisii* even though *C. sapidus* larvae were exposed to daily changes of 0.02 ppm malathion from 46 to 52 days as compared to an exposure of 17 days in the case of *R. harrisii* larvae. The sensitivity of *R. harrisii* and *C. sapidus* larvae to methoxychlor was just the opposite that observed to the sensitivity to malathion. Larvae of each species were about equally sensitive to concentrations of mirex in a range from 0.01 ppb to 10.0 ppb.

If residues of malathion of 0.8 to 3.2 ppm can remain in the water after aerial application of 256 g/ha as Conte and Parker (1971) reported, these concentrations would be lethal to *R. harrisii* and *C. sapidus* larvae.

Although it is known that hydrolysis increases as alkalinity does (Walker and Stojanovic, 1973), the half-life of 0.5 ppm malathion in water is reported to be about one month at pH 8 and 28°C (EPA-540/1-75-005). These are similar to the conditions in which *R. harrisii* and *C. sapidus* larvae develop and further supports the belief that sprays of malathion for mosquito control could be hazardous to crab larvae.

Sublethal effect

The average duration of zoeal development and total time from hatching to 1st crab stage were shortest in acetone control and were lengthened with each increase in concentration of malathion in *R. harrisii* from 0.011 ppm to 0.02 ppm (Table 23) and in *C. sapidus* from 0.02 ppm to 0.11 ppm (Table 30). The variation in duration of larval development of *R. harrisii* is related statistically to stages ($P < 0.001$) and media ($P < 0.05$) (Table 24), whereas the variation in duration of larval development of *C. sapidus* is related to stages, media and the interaction of the two at the 0.001 level of probability.

Sexual morphogenesis

No modification of the structure of the genital apparatus of *R. harrisii* and *C. sapidus* was related to increase of concentrations of methoxychlor or malathion. The anlage of germinal cells, protogonia, develop as in crabs reared in acetone control. The genital ducts and androgenic gland differentiate progressively in the males, as well as the spermatheca and vagina in the females. The onset of oogenesis takes place earlier than spermatogenesis, just as it does in the controls. No particular effect of insecticides has been no-

ticed during the process of gametogenesis in the male or female.

Though not associated with sexual morphogenesis it was noticed that 0.017 ppm and 0.02 ppm malathion induces autotomy of legs at the megalopa stage, as well as in crab stages, of *R. harrisii*. This occurrence does not prevent the megalopa from molting to the crab stage. It is known that autotomy is usually associated with mechanical stimulation of legs of crabs. In this case, a poisonous fluid, malathion, induces autotomy as early as the megalopa stage.

Mortality

The main effects of malathion on the mortality of four zoeal stages and a megalopa stage of *R. harrisii* and eight zoeal stages and a megalopa stage of *C. sapidus* are related in a highly significant way to stages, media and interaction of the two (Tables 26 and 33).

A single classification ANOVA of mortality data from each medium in Table 25 and a calculation of the least significant difference, shows that there isn't any single stage of *R. harrisii* which is significantly different ($t_{0.01}$) in mortality from any other in acetone control. Larvae in zoeal stage II and megalopa were significantly sensitive ($t_{0.01}$) to 0.011 ppm malathion, as were larvae in zoeal stage II to 0.014 ppm, 0.017 ppm and 0.02 ppm, and larvae in zoeal stage I to 0.05 ppm.

In *C. sapidus*, no stages were significantly sensitive to 0.02 ppm malathion. Larvae in zoeal stages II and III were significantly sensitive ($P < 0.05$) to 0.05 ppm malathion, as were larvae in zoeal stages I, II and III to 0.08 ppm and larvae in stage I to 0.11 ppm malathion.

An examination of the percent mortality of larvae in developmental stages of *C. sapidus* in Table 32 shows that more larvae in the first zoeal stage died in every replicate of 0.11 ppm malathion than in any replicate of the other concentrations of malathion. It is believed, therefore, that the concentration of 0.11 ppm prepared by Hazleton Laboratories America, Inc., is higher than 0.08 ppm, even though the total mortality to 1st crab was slightly higher in 0.08 ppm than 0.11 ppm malathion.

Many acute toxicity tests, but few chronic bioassays have been made to determine the effect of malathion on aquatic or-

ticed during the process of gametogenesis in the male or female.

Though not associated with sexual morphogenesis it was noticed that 0.017 ppm and 0.02 ppm malathion induces autotomy of legs at the megalopa stage, as well as in crab stages, of *R. harrisii*. This occurrence does not prevent the megalopa from molting to the crab stage. It is known that autotomy is usually associated with mechanical stimulation of legs of crabs. In this case, a poisonous fluid, malathion, induces autotomy as early as the megalopa stage.

Mortality

The main effects of malathion on the mortality of four zoeal stages and a megalopa stage of *R. harrisii* and eight zoeal stages and a megalopa stage of *C. sapidus* are related in a highly significant way to stages, media and interaction of the two (Tables 26 and 33).

A single classification ANOVA of mortality data from each medium in Table 25 and a calculation of the least significant difference, shows that there isn't any single stage of *R. harrisii* which is significantly different ($t_{0.01}$) in mortality from any other in acetone control. Larvae in zoeal stage II and megalopa were significantly sensitive ($t_{0.01}$) to 0.011 ppm malathion, as were larvae in zoeal stage II to 0.014 ppm, 0.017 ppm and 0.02 ppm, and larvae in zoeal stage I to 0.05 ppm.

In *C. sapidus*, no stages were significantly sensitive to 0.02 ppm malathion. Larvae in zoeal stages II and III were significantly sensitive ($P < 0.05$) to 0.05 ppm malathion, as were larvae in zoeal stages I, II and III to 0.08 ppm and larvae in stage I to 0.11 ppm malathion.

An examination of the percent mortality of larvae in developmental stages of *C. sapidus* in Table 32 shows that more larvae in the first zoeal stage died in every replicate of 0.11 ppm malathion than in any replicate of the other concentrations of malathion. It is believed, therefore, that the concentration of 0.11 ppm prepared by Hazleton Laboratories America, Inc., is higher than 0.08 ppm, even though the total mortality to 1st crab was slightly higher in 0.08 ppm than 0.11 ppm malathion.

Many acute toxicity tests, but few chronic bioassays have been made to determine the effect of malathion on aquatic or-

ticed during the process of gametogenesis in the male or female.

Though not associated with sexual morphogenesis it was noticed that 0.017 ppm and 0.02 ppm malathion induces autotomy of legs at the megalopa stage, as well as in crab stages, of R. harrisii. This occurrence does not prevent the megalopa from molting to the crab stage. It is known that autotomy is usually associated with mechanical stimulation of legs of crabs. In this case, a poisonous fluid, malathion, induces autotomy as early as the megalopa stage.

Mortality

The main effects of malathion on the mortality of four zoeal stages and a megalopa stage of R. harrisii and eight zoeal stages and a megalopa stage of C. sapidus are related in a highly significant way to stages, media and interaction of the two (Tables 26 and 33).

A single classification ANOVA of mortality data from each medium in Table 25 and a calculation of the least significant difference, shows that there isn't any single stage of R. harrisii which is significantly different ($t_{0.01}$) in mortality from any other in acetone control. Larvae in zoeal stage II and megalopa were significantly sensitive ($t_{0.01}$) to 0.011 ppm malathion, as were larvae in zoeal stage II to 0.014 ppm, 0.017 ppm and 0.02 ppm, and larvae in zoeal stage I to 0.05 ppm.

In C. sapidus, no stages were significantly sensitive to 0.02 ppm malathion. Larvae in zoeal stages II and III were significantly sensitive ($P < 0.05$) to 0.05 ppm malathion, as were larvae in zoeal stages I, II and III to 0.08 ppm and larvae in stage I to 0.11 ppm malathion.

An examination of the percent mortality of larvae in developmental stages of C. sapidus in Table 32 shows that more larvae in the first zoeal stage died in every replicate of 0.11 ppm malathion than in any replicate of the other concentrations of malathion. It is believed, therefore, that the concentration of 0.11 ppm prepared by Hazleton Laboratories America, Inc., is higher than 0.08 ppm, even though the total mortality to 1st crab was slightly higher in 0.08 ppm than 0.11 ppm malathion.

Many acute toxicity tests, but few chronic bioassays have been made to determine the effect of malathion on aquatic or-

ganisms. It is difficult, therefore, to compare our results with those of other investigators. If the concentration of malathion necessary to immobilize or kill 50% of the organisms being tested for 24, 48 or 96 hours is lower than observed in our chronic bioassays with *R. harrisii* and *C. sapidus* larvae, it can be assumed that crab larvae are more resistant. The following common freshwater organisms fit into this category. *Daphnia magna* is immobilized in 50 hours by 0.9 μg/l malathion when kept at 68°F, and *Simocephalus serralatus* is immobilized by 6.2 μg/l in 48 hours at 70°F. Increase in temperature from 50°F to 70°F resulted in an eight-fold decrease in toxicity to the latter species (Sanders and Cope, 1966). The 96-hour TL_m for *Gammarus lacustris* was 0.002 ppm (Gaufin *et al*., 1965) and the 24-hour LC_{50} for larval *Chironomus tentans* was also 0.002 ppm (Karnak and Collins, 1974). The 24-hour LC_{90} for larval mosquitoes, *Culex pipiens quinquefasciatus* ranged from 0.05 to 0.120 ppm in polluted water and 0.100 to 0.240 ppm in tap water (Lewallen and Wilder, 1963).

No studies have been made on the short or long-term effects of malathion on larval decapods, but Eisler (1969) made acute toxicity bioassays of three species of marine decapods. He found that the 96-hour LC_{50} values of malathion to be 33 μg/l for the sand shrimp, *Crangon septemspinosa*, 82 μg/l for grass shrimp, *Palaemonetes vulgaris*, and 83 μg/l for the hermit crab, *Pagurus longicarpus*.

These concentrations are within the range of media used in *C. sapidus* development but are higher than those employed in the *R. harrisii* experiment.

Eisler (1969) was of the opinion that crustaceans are much more sensitive to most organophosphorus insecticides than fish. This may be true for many fish but not for the bluegill, *Lepomis macrochiris*. Concentrations of 66 and 28 μg/l malathion were lethal to all bluegills within 16 and 54 days, respectively. Reproduction and early fry were unaffected by 7.4 μg/l malathion which crippled adult fish after several months of exposure. The safe concentration of malathion to bluegills was experimentally determined to be 3.6 μg/l and the unsafe concentration to be 7.4 μg/l. The maximum acceptable concentration is between the two (Eaton, 1970). It would appear, therefore, that *C. sapidus* larvae are about as sensitive to malathion as bluegills, but *R. harrisii* larvae are more sensitive than bluegills.

SECTION VIII

GENERAL DISCUSSION

ECOLOGICAL IMPLICATIONS

Before considering the possible effects of different concentrations of mirex, methoxychlor and malathion on the complete development of *R. harrisii* and *C. sapidus* from hatching to 1st crab stage in the field, it is important to know in what range of salinities and temperatures normal development of these bioassay crustaceans will take place. Costlow *et al.* (1966) reported that *R. harrisii* can develop from hatching to 1st crab stage in the laboratory in salinities from 2.5‰ to 40‰, but that the best survival, 66% to 90%, will take place in salinities from 15‰ to 25‰. In lower and higher salinities, there is a reduction in survival. In salinities below 15‰, there is higher survival in 30°C than in 20°C, but in salinities from 15‰ to 40‰ survival is higher at lower temperatures. In the Newport River Estuary, N.C., Pinschmidt (1963) collected *R. harrisii* larvae when water temperatures were between 16°C and 34°C, with the greatest abundance at the higher temperatures. Stage I zoeae through stage IV zoeae were present in the plankton in salinities between 0 and 33‰, with the range of greatest abundance between 5‰ and 21‰. The megalopa were collected at salinities between 7‰ and 19‰.

Callinectes sapidus will develop in the laboratory from hatching to the 1st crab stage in salinities from 20‰ to 35‰ and in temperatures from 20°C to 30°C. Zoeae of *C. sapidus* were found in the plankton of the Newport River Estuary in salinities from 18‰ to 36‰ and temperatures from 16°C to 31°C. Megalopa were collected in salinities from 19‰ to 32‰ and in waters between 25°C and 30°C (Pinschmidt, 1963). The megalopa in the laboratory, however, can withstand salinities from 5‰ to 40‰ and temperatures of 15°C to 30°C (Costlow, 1967).

Mirex

For mirex to affect blue crab larvae directly, fire ant bait or leached mirex would have to be in water from 18‰ to 35‰ salinity. Blue crab zoeae are so small that they could not eat a flake of mirex bait. In our opinion, zoeae could be affected by mirex which leaches out of the bait, provided they settled on or near the bait between spurts of swimming. Larvae of later zoeal stages are known to sink further down in the water column than do larvae of earlier stages. Ludke et al. (1971) reported that 19 out of 20 crayfish died in seven days from mirex which leached from 4X mirex bait. In a similar experiment 33 of 35 crayfish died after 54 hours. Water samples contained 0.86 ppb mirex and three pooled samples of crayfish had body residues of 1.602, 1.409 and 1.339 ppm mirex. In this investigation it was determined that 0.01 ppb and 0.1 ppb mirex were sublethal concentrations and that 1.0 ppb was an acutely toxic concentration. It is probable, therefore, that a concentration of 0.86 ppb or lower could cause sublethal effects and mortality of blue crab larvae in the field. Additional mirex could be obtained through the food chain. Naqvi and de la Cruz (1973) reported the presence of mirex in freshwater oganisms which did not receive direct mirex treatment. This indicates widespread movement of mirex in the environment and a spread of mirex through the food chain. The resistance of mirex to degradation in the environment and its storage unaltered in animals favors bioaccumulation (Metcalf et al., 1973).

Megalopa, in our opinion, are much more likely to be affected directly and indirectly by mirex bait than are zoeae. It is possible that they may nibble on mirex bait in the field, as they do in the laboratory. They are also more apt to come in contact with mirex which leaches from the bait, because they often crawl or swim near the bottom. They are capable of eating more and larger organisms than zoeae and thus obtain more mirex through the food chain. Since megalopa are often found in the lower part of the water column, they are in a position to be carried landward in estuaries as Bousfield (1955) reported for late-stage barnacles. Pinschmidt (1963) and others have found blue crab megalopa throughout most of the 17 mile Newport River Estuary, N. C. Being more widely distributed in an estuary, they are more apt to be affected by mirex than zoeae which are confined to waters of a salinity of 20‰ and above.

Methoxychlor

As a substitute insecticide for DDT, methoxychlor is nearly as toxic to target organisms as DDT, yet it is biodegradable. Hence, it does not give rise to such long lasting residues as DDT (Burdick *et al.*, 1968; Metcalf *et al.* 1971). Although most of the literature on methoxychlor effects concern freshwater organisms, Henderson *et al.* (1959) concluded that pH and alkalinity have no major effect on toxicity of fish, and so it is reasonable to assume that the insecticide is not any less toxic to marine than freshwater organisms.

It is not known what concentrations of methoxychlor would have sublethal and lethal effects on larval development of *R. harrisii* and *C. sapidus* in the field. One can only make the following postulations based on the current investigation and on incomplete information in the literature.

It is probable that methoxychlor would have similar effects on the complete development of *R. harrisii* and *C. sapidus* in the field to those described in the laboratory if the salinity and temperature of the water were favorable for complete development. Continuous exposure to 1.0 ppb methoxychlor would have little effect on the complete development of the mud-crab, *R. harrisii*, from hatching to the 1st crab stage. It is probable that continuous exposure to concentrations of 2.5 ppb to 5.5 ppb would reduce survival markedly and exposure to 7.0 ppb would be lethal. *C. sapidus* larvae, on the other hand, would be much more sensitive to lower concentrations of methoxychlor than *R. harrisii*. Concentrations of 0.7 ppb to 1.0 ppb would probably reduce survival of larvae to the 1st crab stage and concentrations of 1.3 ppb to 1.9 ppb would be acutely toxic.

The effect of methoxychlor on *R. harrisii* and *C. sapidus* larvae would also depend upon the rate of breakdown of the insecticide, its dilution in the estuary and bioaccumulation. There is a difference of opinion as to how long methoxychlor will remain in the water. Bedford *et al.* (1968), using freshwater mussels as monitors of river water, reported residues from spraying for the Dutch elm disease after two weeks equal to residues at ten weeks and peak concentrations at six weeks. Kennedy *et al.* (1970) made residue analyses of bluegills in ponds which had been treated with 0.01 ppm methoxychlor. After one day the fish had residues of 2.11 ppm and 2.78 ppm, after seven days the residues peaked at 3.35 ppm and 4.0 ppm, and after 56 to 84 days there were no detectable residues. Fish from ponds treated with 0.04 ppm methoxychlor had residues of 9.80 ppm and 13.90 ppm at day one, peaked with residues of 20.60 ppm and 21.10 ppm at day three and had no residues at 56 and 84 days.

If methoxychlor can remain in river water for ten weeks, as Bedford *et al*. (1968) reported, it could be carried by river water from a recently sprayed area to an estuary. In the estuary, it might have sublethal and/or highly toxic effects on *R. harrisii* and *C. sapidus* larvae, especially if repeated applications of the insecticide were used. The Florida estuary from which we obtained ovigerous *R. harrisii* for malathion experiments in January and February, 1975, must have been such an area, for these crabs had residues of methoxychlor ranging from 0.57 ppm to 5.50 ppm. In this environment both species could obtain methoxychlor from water and by way of the food chain. Larvae of *C. sapidus* would probably be affected before larvae of *R. harrisii*, not only because they accumulate residues at a faster rate than *R. harrisii*, but because they reach higher peaks.

Malathion

The toxicity of malathion to aquatic organisms apparently depends upon the exposure time, temperature and pH of the water and the species. Malathion decomposes at high temperatures and is susceptible to hydrolysis as alkalinity increases. Its residues are short-lived (Walker and Stojanovic, 1973; EPA-540/1-75-005). Even though malathion is biodegradable, it may be highly toxic to target and non-target organisms alike. Since it is a non-persistent insecticide, repeated applications of malathion may be necessary for control of pests, and cumulative reduction of acetylcholinesterase may occur (Coppage and Matthews, 1974). Furthermore there is documented evidence from the literature that arthropod pests develop resistance to malathion (Mengle and Lewallen, 1963; Busvine, 1959; Brown and Abedi, 1960; LaBrecque and Wilson, 1960; Mount, Seawright and Pierce, 1974). Hence, increasing concentrations of malathion have to be used to control pests and the danger to non-target organisms becomes greater.

Few laboratory and field studies have been conducted on the effects of malathion on estuarine animals, and, as far as known, there have been on publications on the effects of malathion on the larval development of crabs.

Tagatz *et al*. (1974) found that thermal fogging [420 g/ha (6 wt oz/acre)] and ULV aerosol spraying [57 g/ha (0.64 fl oz)] left residues in marsh water as high as 5.2 ppb and 0.49 ppb, respectively. Since *R. harrisii* development is affected by concentrations from 11.0 ppb to 20.0 ppb malathion and *C. sapidus* larvae by concentrations from 20.0 ppb to 110.0 ppb, the residues Tagatz *et al*. (1974) found in the water would probably not have any direct effect on either *R. harrisii* or

C. sapidus larvae. The initial spraying and bioaccumulation had no effect on confined blue crabs, grass shrimps, pink shrimp or sheepshead minnow (Tagatz *et al.*, 1974), but could have affected crab larvae. Conte and Parker (1971) reported that aerial application of 256 g/ha (3 fl oz/acre) to marsh embayments in Texas killed 14 to 80% of commercial shrimp, *Penaeus aztecus* and *P. setiferous*. They found residues of malathion of 0.8 ppm to 3.2 ppm in the water 48 hours after spraying. These concentrations of malathion would undoubtedly kill the larvae of *R. harrisii* and *C. sapidus* for they are above the range of concentrations which were found to be acutely toxic to each species.

SECTION IX

REFERENCES

Alley, E. G. 1973. The use of mirex in control of the imported fire ant. J. Environ. Quality. 2(1):52-61.

Bedford, J. W., E. W. Roelofs, and M. J. Zabik. 1968. The freshwater mussel as a biological monitor of pesticide concentrations in a lotic environment. Limnol. Oceanogr. 13(1):118-126.

Bookhout, C. G., A. J. Wilson, Jr., T. W. Duke, and J. I. Lowe. 1972. Effects of mirex on the larval development of two crabs. Water Air Soil Pollut. 1:165-180.

Bookhout, C. G., and J. D. Costlow, Jr. 1975. Effects of mirex on the larval development of blue crab. Water Air Soil Pollut. 4:113-126.

Borthwick, P. W., T. W. Duke, A. J. Wilson, Jr., J. I. Lowe, J. M. Patrick, Jr., and J. C. Oberheu. 1973. Accumulation and movement of mirex in selected estuaries of South Carolina, 1969-71. Pestic. Monit. J. 7(1):6-26.

Bousfield, E. L. 1955. Ecological control of the occurrence of barnacles in the Miramichi Estuary. Bull. Nat. Mus. Canada (Ottawa). 137. 69 p.

Brown, A. W. A., and Z. H. Abedi. 1960. Cross-resistance characteristics of a malathion-tolerant strain developed in *Aedes aegypti*. Mosquito News. 20(2):118-124.

Buchanan, D. V., R. E. Milleman, and N. E. Stewart. 1970. Effects of the insecticide Sevin on various stages of the dungeness crab, *Cancer magister*. J. Fish. Res. Bd. Can. 27:93-104.

Burdick, G. E., H. J. Dean, E. J. Harris, J. Skea, C. Frisa, and C. Sweeney. 1968. Methoxychlor as a blackfly larvicide, persistence of its residues in fish and its effect on stream arthropods. N.Y. Fish Game J. 15(2):121-142.

Busvine, J. R. 1959. Patterns of insecticide resistance to organophosphorus compounds in strains of houseflies from various sources. Entomologia exp. appl. (Amsterdam). *2*:58-67.

Churchill, E. P. 1919. Life history of the blue crab. Bull U. S. Bur. Fish. *36*:91-128.

Conte, F. S., and J. C. Parker. 1971. Ecological aspects of selected crustacea of two marsh embayments of the Texas coast. Texas A. and M. Univ., Sea Grant Publ. No TAMU-SG-71-211. 184 p.

Coon, D. W., and R. R. Fleet. 1970. The ant war. Environment. *12*(10):28-38.

Coppage, D. L., and T. W. Duke. 1971. Effects of pesticides in estuaries along the Gulf and Southeast Atlantic Coasts. In: Proceeding of the 2nd Gulf Coast Conference on Mosquito Suppression and Wildlife Management, Schmidt, C. H. (ed.). Washington, D. C. p. 24-31.

Coppage, D. L., and E. Matthews. 1974. Short-term effects of organophosphate pesticides on cholinesterases of estuarine fishes and pink shrimp. Bull. Environ. Contam. Toxicol. *11*(5):483-488.

Costlow, J. D. 1967. The effect of salinity and temperature on survival and metamorphosis of megalops of the blue crab *Callinectes sapidus*. Helgoländer Wiss. Meeresunters (Kiel). *15*:84-97.

Costlow, J. D., Jr., and C. G. Bookhout. 1959. The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. Biol. Bull. *116*(3):373-396.

Costlow, J. D., Jr., and C. G. Bookhout. 1960. A method for developing brachyuran eggs *in vitro*. Limnol. Oceanogr. *5*(2):212-215.

Costlow, J. D., Jr., C. G. Bookhout, and R. J. Monroe. 1966. Studies on the larval development of the crab, *Rhithropanopeus harrisii* (Gould). I. The effect of salinity and temperature on larval development. Physiol. Zool. *39*(2):81-100.

Courtenay, W. R., Jr., and M. H. Roberts, Jr. Environmental effects on toxaphene toxicity to selected fishes and crustaceans. Environmental Protectional Agency. Washington, D. C. EPA-R3-73-035. April 1973. 73 p.

Darsie, R. F., Jr., and F. E. Corriden. 1959. The toxicity of malathion to killifish (Cyprinodontidae) in Delaware. J. econ. Ent. 52(4):696-700.

Eaton, J. G. 1970. Chronic malathion toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Wat. Res. 4(10): 673-684.

Eisler, R. 1969. Acute toxicities of insecticides to marine decapod crustaceans. Crustaceana. 16:302-310.

Epifanio, C. E. 1971. Effects of dieldrin in seawater on the development of two species of crab larvae, *Leptodius floridanus* and *Panopeus herbstii*. Mar. Biol. 11(4):356-362.

Epifanio, C. E. 1973. Dieldrin uptake by larvae of the crab *Leptodius floridanus*. Mar. Biol. 19:320-322.

Gaufin, A. R., L. D. Jensen, A. V. Nebeker, T. Nelson, and R. W. Teel. 1965. The toxicity of ten organic insecticides to various aquatic invertebrates. Water Sewage Works. 112:276-279.

Henderson, C., Q. H. Pickering, and C. M. Tarzwell. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Trans. Amer. Fish. Soc. 88:23-32.

Initial scientific and minieconomic review of malathion. Environmental Protection Agency. Washington, D. C. EPA-540/1-75-005. March 1975. 14 p.

Jernelöv, A., R. Rosenberg, and S. Jensen. 1972. Biological effects and physical properties in the marine environment of aliphatic chlorinated by-products from vinyl chloride production. Wat. Res. (London). 6:1181-1191.

Johnson, B. T., and J. O. Kennedy. 1973. Biomagnification of p,p'-DDT and methoxychlor by bacteria. Appl. Microbiol. 26(1):66-71.

Karnak, R. E., and W. J. Collins. 1974. The susceptibility to selected insecticides and acetylcholinesterase activity in a laboratory colony of midge larvae, *Chironomus tentans* (Diptera: Chironomidae). Bull. Environ. Contam. Toxicol. 12(1):62-69.

Kennedy, H. D., L. L. Eller, and D. F. Walsh. 1970. Chronic effects of methoxychlor on bluegills and aquatic invertebrates. U. S. Bur. Sport Fish. Wildl. Technical Paper No. 53. 18 p.

LaBrecque, G. C., and H. G. Wilson. 1960. Effect of DDT resistance on the development of malathion resistance in house flies. J. econ. Ent. 53(2):320-321.

Lee, J. H., C. E. Nash, and J. R. Sylvester. Effects of mirex and methoxychlor on striped mullet, *Mugil cephalus* L. Environmental Protection Agency. Corvallis, Oregon. EPA-660/3-75-015. May 1975. 18 p.

Lewallen, L. L., and W. H. Wilder. 1963. Laboratory tests of insecticides on mosquito larvae in polluted and tap water. J. econ. Ent. 56(6):834-835.

Lowe, J. I., P. R. Parrish, A. J. Wilson, Jr., P. D. Wilson, and T. W. Duke. 1971. Effects of mirex on selected estuarine organisms. In: Proceedings of the 36th N. Amer. Wild. Nat. Resources Conf., Portland, Oregon. p. 171-186.

Ludke, J. L., M. T. Finley, and C. Lusk. 1971. Toxicity of mirex to crayfish, *Procambarus blandingi*. Bull. Environ. Contam. Toxicol. 6(1):89-95.

MacFarlane, J., T. Dirks, and S. Uk. 1975. Symptoms of mirex, dieldrin, and DDT poisoning in the field cricket, *Gryllus pennsylvanicus* Burmeister, and effect on activity of the central nerve cord. Pestic. Biochem. Physiol. 5:57-64.

Mahood, R. K., M. D. McKenzie, D. P. Middaugh, S. J. Bollar, J. R. Davis, and D. Spitsbergen. 1970. A report on the cooperative blue crab study - South Atlantic States. Fla. Dept. of Nat. Resour., Div. Mar. Res., Contribution Series No. 139., Study in cooperation with the U. S. Dept. of Interior, Bureau of Commercial Fisheries. 32 p.

McKenzie, M. D. 1970. Fluctuations in abundance of the blue crab and factors affecting mortalities. S. Carol. Wildl. Res. Dept., Mar. Resour. Div., Tech. Rep. 45 p.

Mengle, D. C., and L. L. Lewallen. 1963. Metabolism of malathion by a resistant and a susceptible strain of *Culex tarsalis*: I. Degradation *in vivo* and identification of organic soluble metabolites. Mosquito News. *23*(3): 226-233.

Merna, J. W., M. E. Bender, and J. R. Novy. 1972. The effects of methoxychlor on fishes. I. Acute toxicity and breakdown studies. Trans. Amer. Fish. Soc. *101*:298-301.

Merna, J. W., and P. J. Eisele. The effects of methoxychlor on aquatic biota. Environmental Protection Agency, Washington, D. C. EPA-R3-73-046. Sept. 1973. 59 p.

Metcalf, R. L., G. K. Sangha, and I. P. Kapoor. 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Tech. *5* (8):709-713.

Metcalf, R. L., I. P. Kappor, P. Lu, C. K. Schuth, and P. Sherman. 1973. Model ecosystem studies of the environmental fate of six organochlorine pesticides. Environ. Health Perspectives. *4*:35-44.

Mount, G. A., J. A. Seawright, and N. W. Pierce. 1974. Selection response and cross-susceptibility of a malathion-resistant strain of *Aedes taeniorhynchus* (Wiedemann) to other adulticides. *Mosquito News. 34(3)*:276-277.

Naqvi, S. M., and A. A. de la Cruz. 1973. Mirex incorporation in the environment: Residues in nontarget organisms - 1972. Pestic. Monit. J. *7*(2):104-111.

O'Brien, R. D. 1967. Insecticides. New York, Academic Press. 332 p.

Pinschmidt, W. C., Jr. 1963. Distribution of crab larvae in relation to some environmental conditions in the Newport River Estuary, North Carolina. Manuscr. Duke Univ. (unpubl.). 112 p.

Plapp, F. W. 1973. Mirex: Toxicity, tolerance, and metabolism in the house fly (*Musca domestica* L.). Environ. Entomol. *2*:1058-1061.

Redmann, G. 1973. Studies on the toxicity of mirex to the estuarine grass shrimp, *Palaemonetes pugio*. Gulf Res. Rep. *4*(2):272-277.

Sanders, H. O., and O. B. Cope. 1966. Toxicities of several pesticides to two species of Cladocerans. Trans. Am. Fish. Soc. *95*:165-169.

Tagatz, M. E., P. W. Borthwick, G. H. Cook, and D. L. Coppage. 1974. Effects of ground applications of malathion on salt-marsh environments in northwestern Florida. Mosquito News. 34(3):309-315.

Tagatz, M. E., P. W. Borthwick, and J. Forester. 1975. Seasonal effects of leached mirex on selected estuarine animals. Arch. Environ. Contam. Toxicol. 3(3)(In press).

U. S. Department of Agriculture. 1968. Suggested guide for the use of insecticides to control insects affecting crops, livestock, households, stored products, forests, and forest products. Agriculture Research Service and Forest Service. Agriculture Handbook 331. 273 p.

Walker, W. W., and B. J. Stojanovic. 1973. Microbial versus chemical degradation of malathion in soil. J. Environ. Quality. 2(2):229-232.

Wallner, W. E., N. C. Leeling, and M. J. Zabik. 1969. The fate of methoxychlor applied by helicopter for smaller European elm bark beetle control. J. econ. Ent. 62(5): 1039-1042.

SECTION X

GLOSSARY

acute toxicity tests: short-term exposure to concentrations of toxicant which will be lethal to 50% of the larvae in a short interval of time - 24 h, 48 h, or 96 h.

acutely toxic concentrations: concentrations of insecticide in which 10% of the larvae or less survive to the 1st crab stage.

ANOVA: analysis of variance.

chronic tests: long-term exposure to toxicant.

differential survival: reduction of survival with each increase in insecticide.

external sex characters: secondary sex characters which differ in male and female crab stages, such as pleopods.

first crab stage: first stage after molt from megalopa; has adult morphology with abdomen bent under cephatothorax, but is sexually immature.

g/ha: grams per hectare.

h: hour.

internal sex characters: development of gonads and their ducts.

juvenile: crab stages which have adult morphology but are sexually immature.

megalopa: stage of development of a crab between last zoeal stage and 1st crab stage; is dorso-ventrally depressed; has all cephalothoracic and abdominal appendages present and functional; and has extended abdomen.

μg/g: micrograms per gram = parts per million.

μg/l: micrograms per liter = parts per billion.

mg/l: milligrams per liter = parts per million.

molt: the process of shedding the exoskeleton which is necessary for growth during larval and juvenile development in arthropods, including crustaceans.

non-target organism: organism which is not an intended target of an insecticide.

pleopods: paired abdominal appendages which are functional in megalopa, juvenile and adult crab.

ppb: parts per billion.

ppm: parts per million.

ppt: ‰ = parts per thousand.

setae: bristle-like extensions of the exoskeleton.

sublethal concentrations: concentrations of insecticide in which more than 10% of the larvae survive to the 1st crab stage.

sublethal effects: effects which occur in larvae reared in sublethal concentrations, but not in acetone control, and which become more pronounced as concentrations are increased.

target organism: organism which is the intended target of an insecticide.

TL_{50}: tolerance limit; the concentration of toxicant in water at which fifty percent of the animals are able to survive for a specified period of exposure.

zoea(e): a planktotropic larval stage of a crab with a laterally compressed cephalothorax and abdomen, and two thoracic appendages (maxillipeds) for swimming.

zoeal development: refers to all zoeal stages from time of hatching to megalopa stage (i.e., four zoeal stages in *R. harrisii* and seven to eight zoeal stages in *C. sapidus*).